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FLUORESCENT LIGHT INDUCED OXIDATION IN MILKFAT

by

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A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled Fluorescent Light Induced Oxidation in Milkfat submitted by Chan-Thanh Du in partial fulfilment of the requirements for the degree of Master of Science.

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ABSTRACT

The oxidation of milkfat was found to be promoted by narrow visible wavebands isolated from four types of fluorescent lamps with the exception of those at the red end of the visible spectrum. Although major light absorption could be observed only in the region of carotene absorption, milkfat oxidation was promoted by light of all wavebands below 710 nm.

Treatment of milkfat with activated charcoal removed carotene and reduced the capacity of milkfat to be oxidized on exposure to light, except in the blue end of the visible spectrum and in the near ultraviolet. Addition of β-carotene and phospholipids did not restore the normal oxidation pattern.

The existence of substances which play a role in the photochemical oxidation of milkfat and which were removed by activated charcoal was suggested.

INTRODUCTION

Fats and fat-containing foods have a spontaneous tendency to undergo autoxidation. Such oxidation usually results in flavor changes which adversely affect acceptability.

The process of fat oxidation is greatly accelerated in the presence of light. Although the mechanism of this light-induced oxidation in fatty materials has not been fully explained, the accelerating effect of light was noted by investigators at the turn of the century (Browne, 1899 ; Siegfield, 1909.) , and emphasized by recent workers with reference to present marketing conditions (deMan, Rajan and Pimentel , 1965 ; Armstrong , 1967 ; Gilchrist, Vigay and Humbert , 1968.).

Although some near ultraviolet light is emitted from the commonly used fluorescent light tubes, the spectral energy output of these lamps lies substantially in the visible region (300-750 nm) . The substrate of fat oxidation (unsaturated fatty acids) exhibits light absorption in the far ultraviolet range (180 nm) . Even after conjugation in the early stages of autoxidation and the resulting bathochromic shifts, the area of absorption still lies in the near ultraviolet portion of the spectrum (320 nm) . Light-induced oxidation in edible fats is commonly attributed to the presence of ultraviolet radiation and effects resulting from visible light are often considered of little or no consequence (Coe and LeClerc , 1932 ; Pimentel , 1966 ; Gilchrist et al. 1968.) .

It was the purpose of this study to investigate the effects on milkfat oxidation of narrow isolated wavebands of visible and near ultraviolet light from a few fluorescent lamp sources , and to furnish some possible explanations of the experimental results obtained.

REVIEW OF LITERATURE

(A) Effect of light in promoting oxidation of fats.

The pro-oxidant effect of light in fat oxidation is well known . Studies which have shown the deleterious effect of light on milkfat have been reported by many workers (Hunziker and Hosman , 1917 ; Emery and Henley , 1922 ; Briggs , 1932 ; Henderson and Roudhouse , 1934 ; Ritter and Nussbaumer , 1938.).

Studies with different packaging materials have shown that the light transmitted through butter wrappers was the main cause of off-flavor development in retail butter (Lovachev and Kolesnik , 1965 ; Pimentel , 1966 ; Radtke and Heiss, 1967 ; Downey and Murphy , 1968.) . Futschik and Aigner (1955) exposed aluminum foil-wrapped, parchment-wrapped and unwrapped butter to a 40 watt filament lamp and a 30 watt ultraviolet lamp at a temperature of 15 ^{o}C for 24 hours. Taste, smell and appearance of butters wrapped in aluminum foil were satisfactory irrespective of the types of light, but under both lights, the unwrapped samples and those wrapped in parchment showed a marked deterioration in quality. Exposure of parchment wrapped butter on supermarket shelves for one day was reported to be sufficient to cause butter to deteriorate to such an extent that it became unpalatable (deMan et al. 1965.) . The content of carotene, vitamin A and vitamin E were also reported to decrease to significantly low levels during the exposure .

Pimentel (1966) indicated that the extent of oxidation of butter as effected by fluorescent light depends to a great extent on the distance from , and length of time exposed to , the light source . In a recent study (Gilchrist et al. 1968.), it was reported that increased peroxide values in butter were usually detected after about thirty minutes of exposure to fluorescent light. The values were greater in the surface layer than in the second and third one-tenth-inch slices . It was suggested that the main factor contributing to butter oxidation was the direct exposure to light and , with current industrial practices , temperature and storage time contributed very little to butter deterioration .

Several studies have indicated that the oxidation of fats is accelerated more rapidly by the shorter wavelengths of light . Coe and LeClerc (1932) reported that the action of light in the production of rancidity varied with the wavelengths of light . Selective ultraviolet light hastened the development of rancidity as compared with natural sunlight . Using a 100 watt Sun Lamp , McConnel and Esselen (1946) found that amber glass , which excluded most of the incident light below 500 nm , was effective in retarding off-flavor development compared with clear glass . However , amber glass afforded less protection when samples were exposed to direct sunlight .

In a study employing glass filters to isolate certain wavelength bands from white types of fluorescent lamps , it was

shown , as judged by organoleptic evaluation , that the ultraviolet portion of the fluorescent light spectrum had a tremendous effect on the off-flavor development of butter (Scott , 1962) . The ultraviolet portion of the spectrum and visible light of wavelengths shorter than 500 nm were considered to be the main cause of oxidized flavor defects in butter . Exposure to infrared radiation resulted in some melting and a greasy melted appearance , but rarely produced off-flavor.

Aigner and Aust (1955) irradiated sweet-cream butter with fluorescent lamps . Although different types of yellow filters were employed to cut off the near ultraviolet radiation , a loss in keeping quality in all irradiated samples was reported . In a study of the effect of different types of fluorescent lamps on the quality of milk , whipping cream , butter and cheese , (Futschik and Aigner , 1960 .) thirteen types of fluorescent tubes made by four manufacturers giving white and yellow light (200-260 lux) were compared at a distance of 1.5 meters from the products enclosed in a cabinet at 10-12 ^{o}C . The controls were kept in darkness under similar conditions . The changes in the quality of the products were assessed organoleptically after 24 hours and 72 hours of illumination . With butter , white light was found to be the most harmful . Deterioration was prevented by aluminum wrappers whereas parchment-wrapped butter showed marked deterioration .

The accelerating effect of light on fat oxidation

has led to the use of specially constructed , light accelerated test apparatus as a means of evaluating the stability of edible fats and oils (Gudheims, 1943 ; Moser et al. 1965 .) . The advantages of this light test were reported to be the short time required for completion , the reduction of variation by a controlled light source , reproducibility of results and adaptibility to related food products.

(B) Methods for the measurement of fat oxidation.

Peroxide value. The primary oxidation products formed in the course of fat autoxidation are fat hydroperoxides and peroxides . Estimation of these products has long been accepted as one of the most satisfactory means available for following the autoxidation of edible fats. Although hydroperoxides are flavorless substances, they are potential precursors of off-flavor compounds.

Numerous variants of the analytical procedure for the determination of peroxides in fats have been devised. Most of these methods express the degree of oxidative deterioration in terms of peroxide value in milliequivalents of peroxide per unit weight of fat . The most commonly used are iodometric methods and ferric thiocyanate methods . The modified Stamm method and polarographic methods are also used to some extent .

Iodometric methods : Variations and modifications are many for this method . They are all based on the

assumption that potassium iodide and hydroiodic acid , when brought in contact with fat peroxides , liberate iodine stoichiometrically , with two atoms of liberated iodine equivalent to one atom of oxygen . The amount of iodine supposedly liberated by hydroperoxides is used as the criterion for the extent of oxidative reaction . Although normally used as a volumetric method , the iodometric procedure has been modified for colorimetric use (Swoboda and Lea , 1958 .) . Wheeler's iodometric procedure is the most popular one on this continent (Stansby , 1962.) while the method of Lea (1952) is preferred by most British workers .

Ferric thiocyanate method : This is a method based on the oxidation of ferrous to ferric iron , with a colorimetric determination of the ferric iron as the thiocyanate . The method is sensitive and low peroxide content can be detected (Stine et al. 1954 ; Driver , Koch and Salwin , 1963.) . Unfortunately , oxygen present in the solvents during oxidation enters into the reaction and gives a yield of ferric ion greater than the amount of peroxide present . The criticism of this procedure is that it is subject to considerable error and high values as compared with iodometric procedure (Hamm and Hammond , 1967.) . A liquid-flow semiautomated ferrous thiocyanate procedure was recently described (Starkovich and Rougbal , 1969.) for use in monitoring peroxide values of extracted oils .

Modified Stamm method : Like the ferric thiocyanate test , the method is colorimetric and sensitive , using 1,5-diphenylcarbohydrazide as a reagent which yields a red color when acted on by peroxide . Also like the ferric thiocyanate test , oxygen causes interference and may lead to erroneously high values (Hamm et al. 1965.) .

Polarographic method : A linear relationship between wave height and peroxide value in the early stage of oxidation was reported (Lewis and Quackenbush , 1949 .) . Later in a more detailed investigation , Ricciti , Coleman and Willets (1955) reported that peroxides , hydroperoxides , aldehydes , ketones conjugated with a double bond and alpha diketones could be measured polarographically. Kuta and Quackenbush (1960) reported the half wave potentials of various organic peroxides . A polarographic method in measuring peroxides in soybean oil was reported by Kalbag et al. (1955) .

The thiobarbituric acid test : This test involves the measurement of a thiobarbituric-malonaldehyde complex which is a pink pigment formed from 2-thiobarbituric acid and malonaldehyde, the latter is a product formed from the secondary oxidation of polyunsaturated fatty acids . More details about this test are presented in the Appendix where a modified procedure is proposed.

Carbonyl compounds : Measurement of

carbonylic substances in fat is of importance because such materials impart off-flavors to the products. Carbonyl compounds are usually formed by secondary degradation of the first-formed hydroperoxides.

The most widely used procedure is probably the colorimetric 2, 4-dinitrophenylhydrazine procedure of Lappin and Clark (1951) as modified by Berry and McKerrigan (1958) . In this procedure the total content of saturated and unsaturated carbonyls are estimated at two wavelengths.

More recent methods involve prior separation of carbonyls from the oxidized fat . The method proposed by Lea and Swoboda (1958) employed physical separation of volatile carbonyls by vacuum distillation using the short path, cold finger type of molecular still . The separated carbonyls were then estimated by a colorimetric method.

Keith and Day (1963) devised a chromatographic procedure in which a solution of the oxidized fat in benzene was passed through an alumina/2, 4-dinitrophenylhydrazine reaction column which was said to retain the excess reagent and the dicarbonyls present and thereby permit direct estimation of " free " monocarbonyls in the eluate without previous separation by distillation. Measurement of the absorption at 430, 460 and 480 nm in alkaline solution permited estimation of three main classes of monocarbonyls : alkanals, alk-2-enals and alk-2, 4-dienals .

Schwartz, Haller and Keeney (1963) proposed a chromatographic procedure for the separation and estimation of monocarbonyls using celite-DNPH-phosphoric reaction column . The 2,4-DNPH derivatives were eluted and fractionated, first on magnesia and then on alumina. The monocarbonyl fraction could be purified by absorption on an ion exchange resin. Finally, separation into classes on magnesia and into individual components could be carried out if desired by liquid-liquid partition chromatography . The method was considered by Lea and Jackson (1964) to be promising as a research tool, though rather complicated for routine use.

EXPERIMENTAL

PART 1. EFFECT ON MILKFAT OXIDATION OF ISOLATED WAVEBANDS FROM FLUORESCENT LAMPS

The purpose of these experiments was to assess the effect on milkfat oxidation of different wavebands of light isolated from several types of fluorescent lamps .

Experimental Methods

Three batches of freshly churned butter were obtained from a local manufacturer for experimental use. The first was summer butter and the last two were winter butters. All were free of added annatto coloring matter .

The summer butter was used only in experiments with Cool-White Fluorescent Lamps in Part 1. The carotene content of this butter was approximately three times that of the winter butters.

Sample preparation . To prepare milkfat samples for tests, portions of the butter were melted in a water bath at 45 ^{o}C. Curd and serum were separated by centrifuging at 3,000 rpm for 5 minutes. The oil layer was collected, pooled and tempered again at 45 ^{o}C. Nine-milliliter portions of the tempered oil were pipeted into square holders (1 7/8 x 1 7/8 x 1/2 inches) made from aluminum foil . The product when solidified

formed a piece of milkfat 1 7/8 x 1 7/8 inches and 1/4 inch thick. Samples prepared in this manner were stored in the dark at -25 °C for experimental use.

Light absorbing characteristics of milkfat. Both fresh and light-exposed milkfat samples were dissolved in appropriate solvents for spectroscopic examination. The instruments used for ultraviolet and visible scanning were Perkin-Elmer 202 and Beckman DB-G spectrometers.

Light source. The following types of fluorescent lamps were used in the experiment :

	Types of lamps		Number of tubes per lamp
1.	Cool-White	F48T12CWHO	2
2.	Daylight	F48T12DHO	2
3.	Pink	F40P (Preheat, rapid start)	2
4.	Gold	F40G (Preheat, rapid start)	4

All lamps, except the Cool-White type which had been used for one year, were newly-purchased from the Canadian General Electric Company. The high output light tubes, i.e., Cool-White lamps and Daylight lamps, were mounted parallel to each other 1 1/2 inches apart in a high output type of fixture and lay horizontally on top of a cabinet placed in a room at

5 °C. The Pink and Gold lamps were mounted with an instant - start type of fixture which placed two tubes 5 inches apart.

Light exposure. The prepared milkfat samples in aluminum holders were inserted into 1 7/8 x 1 7/8 x 1/2 inch openings in a wooden box, and a set of filters were fitted snugly on top of the holders 1/8 inch above the samples. The whole box was placed in the light cabinet at a distance of 7 inches from the light source. Covers of the light cabinet were left partially open to avoid accumulation of heat generated from the lamps. Temperature in the vicinity of the samples was 5 ± 1 °C, corresponding to the temperature of the room . Illumination intensity at this level was greater than 500 foot-candle power (under Cool-White Fluorescent Lamp) as measured by a Model No. 213 General Electric Lightmeter. Thermocouple measurement indicated a value of 2 microwatts/cm^2 .

Light filters. A set of ten closely-spaced interference filters, 2 x 2 x 1/8 inches covering the whole range of the visible spectrum were employed in the experiment to obtain narrow, isolated wavebands. In addition, three other glass color filters were used. The transmission characteristics of these filters are shown in Table 1.

Table 1. Transmission Characteristics of Filters

Peak wavelength (nm)	Transmission range (nm)	Peak width at half height (nm)	Transmission (%)
410	377-430	20	35.5
443	424-462	16	40.2
476	464-491	14	30.8
510	492-527	13	33.1
543	527-558	14	38.5
576	559-593	17	40.1
610	587-633	17	39.7
643	616-669	17	38.2
676	649-708	24	42.1
710	685-739	21	40.1
360 *	310-410	55	76
400*	350-520	100	68
800*	760-4500	400	90

The first ten filters comprise the Monopass Filter Set No. 3462, manufactured by Optics Technology Inc., Cal., U.S.A.. They are designed and selected primarily for isolating ten pass bands uniformly spaced through out the visible region.

*The last three are Corning glass color filters, designated C.S. 7-51, 7-59, 7-56 respectively. (Glass Color Filters, CF-3, 1965, Corning Laboratory Products, Corning, N.Y., U.S.A.) .

Measurement of fat oxidation. After various periods of exposure, the samples were analyzed for their peroxide content by the official iodometric procedure of the American Oil Chemists' Society (1964). This method determines, in terms of milliequivalents of peroxide per 1000 grams of sample , all substances which oxidize potassium iodide under the conditions of the test. These are generally assumed to be peroxides or other similar products of fat oxidation.

Three grams of fat were dissolved in 30 ml of glacial acetic acid : chloroform (3 : 2) mixed solvents. One milliliter of saturated potassium iodide was added and mixed by magnetic stirrer for one minute. Thirty milliliter of distilled water and one milliliter of starch indicator were added subsequently, and the whole mixture was titrated with standardized 0.01 N sodium thiosulfate to a colorless end point. The results were expressed as peroxide value (mEq/kg fat) by the following calculation:

$$\text{Peroxide value} = \frac{(a - b) \times N \times 1{,}000}{\text{Weight of sample (g)}}$$

where a = ml of sodium thiosulfate used in the titration of sample.
b = ml of sodium thiosulfate used in the titration of reagent blank.
N = normality of sodium thiosulfate.

Measurement of transmitted light energy. The relative amounts of light transmitted through various filters

were measured with a sensitive thermocouple (a) hooked up with a nanovoltmeter (b). The thermocouple was housed in a black, light-tight wooden case. A three inch diameter concave mirror was placed at the bottom of the case to reflect transmitted light to the thermocouple surface (6.0 x 2.0 mm) which was situated at the mirror's focal point. The light filter was placed on top of the case in an opening of 1 7/8 x 1 7/8 x 1/8 inches. The measuring apparatus is shown schematically in Figure 1. Measurements were carried out at room temperature and voltmeter readings , which were taken five seconds after the instrument was turned on, were converted to energy units (microwatts/ cm^2) by a conversion factor supplied by the thermocouple manufacturer.

(a) Catalog No. RSL-2C, Charles M. Reeder Co., Inc., Detroit, Michigan, U.S.A.

(b) Model 121 Z, Astrodata Inc., Anaheim, Cal., U.S.A.

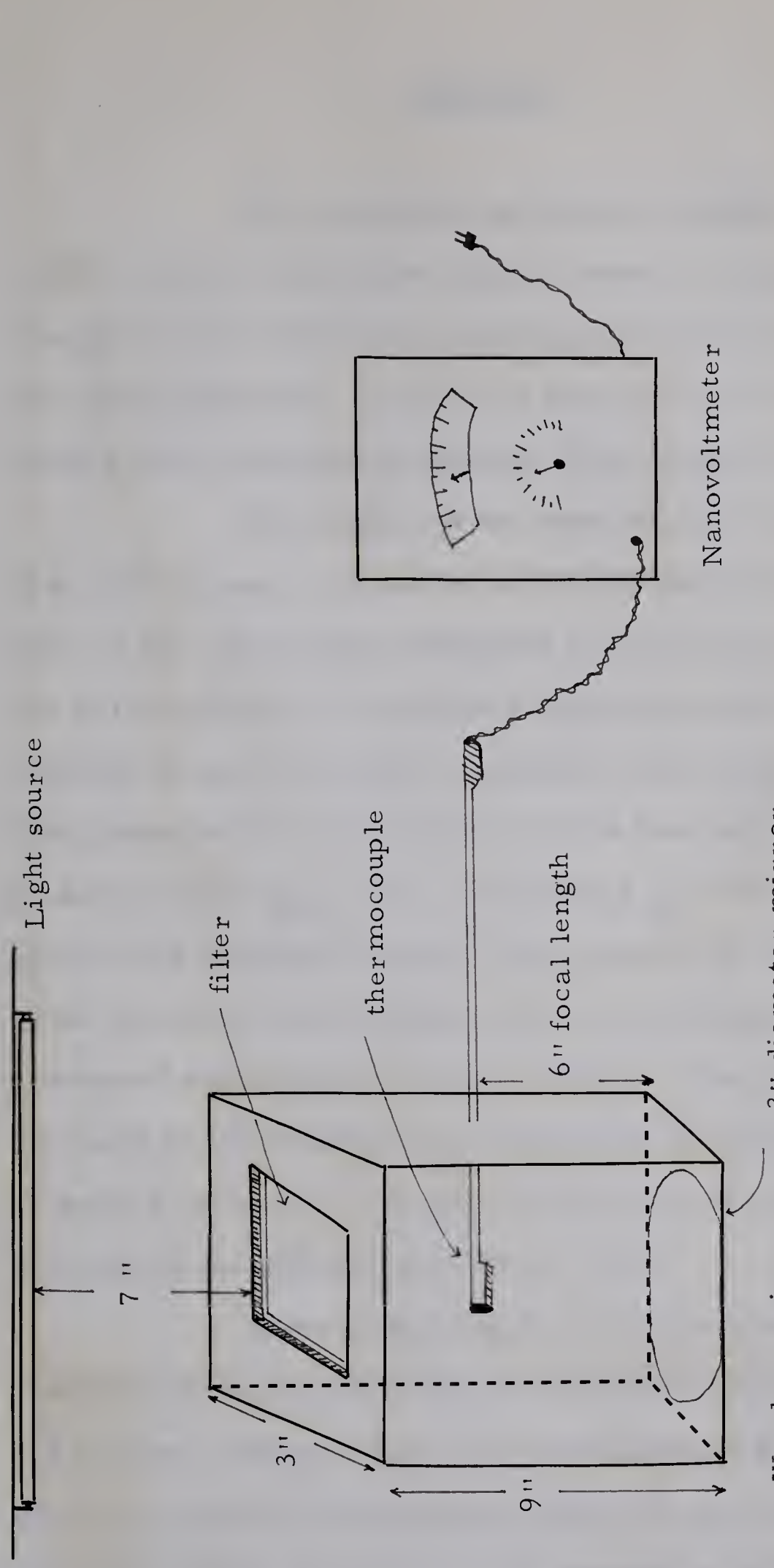


Figure 1. Diagramatic sketch of light detector

RESULTS

The absorption spectrum of milkfat in both the visible and near ultraviolet range is shown in Figure 2. Absorption intensity in the ultraviolet is much greater than in the visible part of the spectrum. Results are expressed in terms of Log $E^{1\%}_{1cm}$ instead of the absorbance obtained from original solution.

The major and the most intense absorption peak is at 230-233 nm, a second is at 270-285 nm and the third is at 302-317 nm. The major absorption at 230-233 nm is probably due to the presence of conjugated materials which have been reported to occur naturally in milkfat (Herb et al. 1962.). This characteristic absorption has also been noted by other workers (Booth et al. 1935 ; Houston et al. 1939.). The presence of conjugated dienes which absorbs at 230-233 nm could also arise from linoleic acid as a preliminary step in the process of autoxidation (Frankel, 1962.) . Absorption at 270-285 nm could be attributable to the absorption of conjugated trienes as well as carotene. Conjugated tetraenes and carbonyls might give rise to the absorption at 302-317 nm.

In the visible portion of the spectrum, the major absorption occurs at 400-500 nm with three maxima at 425, 450 and 480 nm, characteristic of the absorption of β-carotene. There is relatively no absorption from 550 nm toward the red end of the visible spectrum. The intensity of absorption is

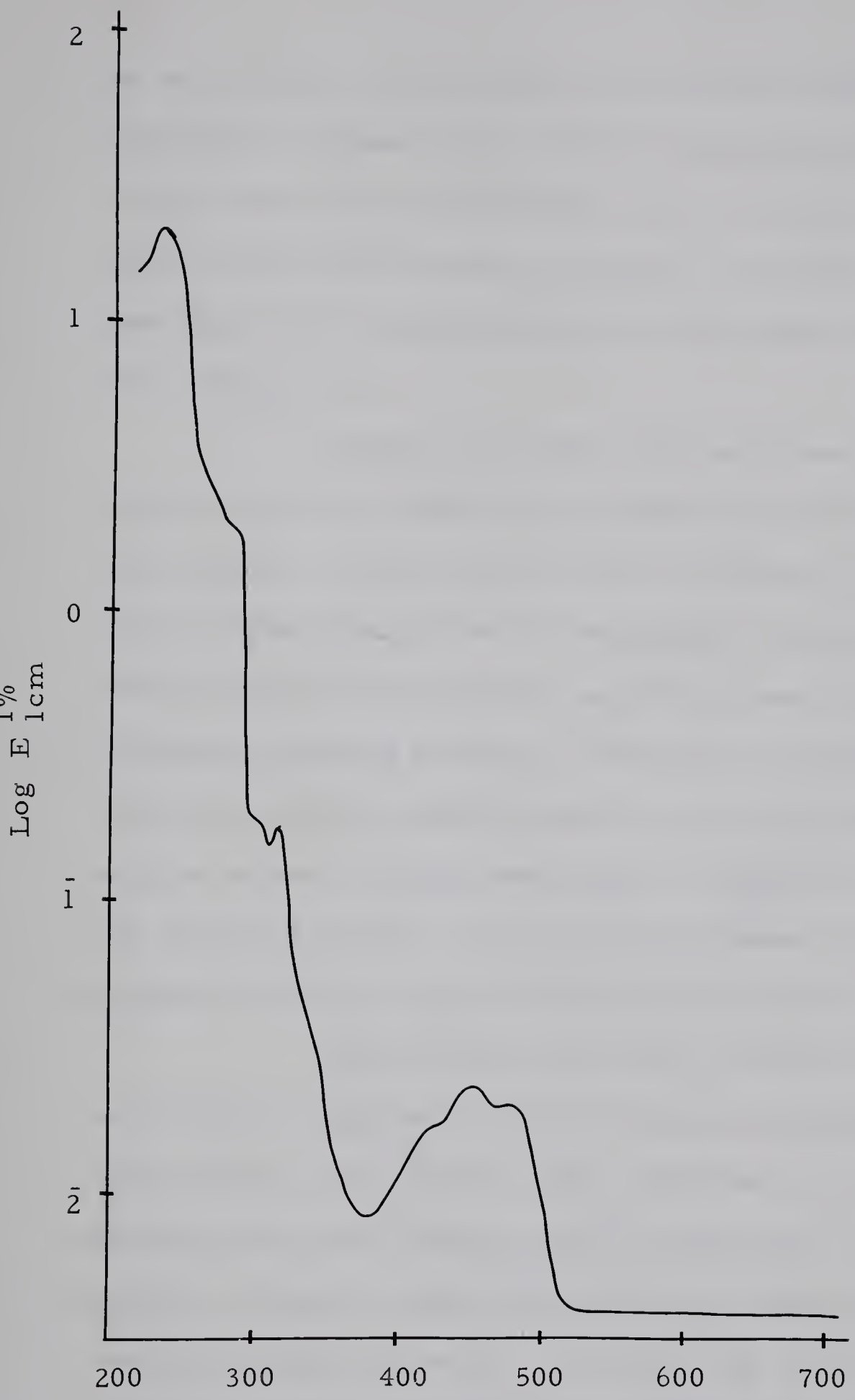


Figure 2. Absorption spectrum of milkfat in cyclohexane

low and remains at a constant level from 540 nm toward longer wavelengths. Exposure of a milkfat sample to the whole spectrum of Cool-White Fluorescent Light caused a decrease in the intensity of the characteristic of carotene absorption without the appearance of a detectable amount of other light-absorbing products (Figure 3).

Results of peroxide determinations on milkfat samples exposed to different wavebands of a Cool-White Fluorescent Lamp for various length of time are shown in Table 2. These results indicated that all wavebands, except those at the infrared end of the spectrum, caused increases in the production of primary oxidation products. The infrared wavebands appear to have no effect on milkfat oxidation and the peroxide value of samples exposed to these wavelengths remained the same as that of control samples. This might be because of the low intensity of infrared radiation from the light source.

Figure 4 shows the effect of different wavelengths and amounts of light on the rate of oxidation in milkfat. When samples were exposed to the whole spectrum (Curve A), oxidation proceeded almost in direct proportion to the time of exposure without any observable induction period. The filter transmitting near ultraviolet, violet and blue light (Curve B) caused the samples to oxidize more rapidly more than the one transmitting only near ultraviolet light (Curve C).

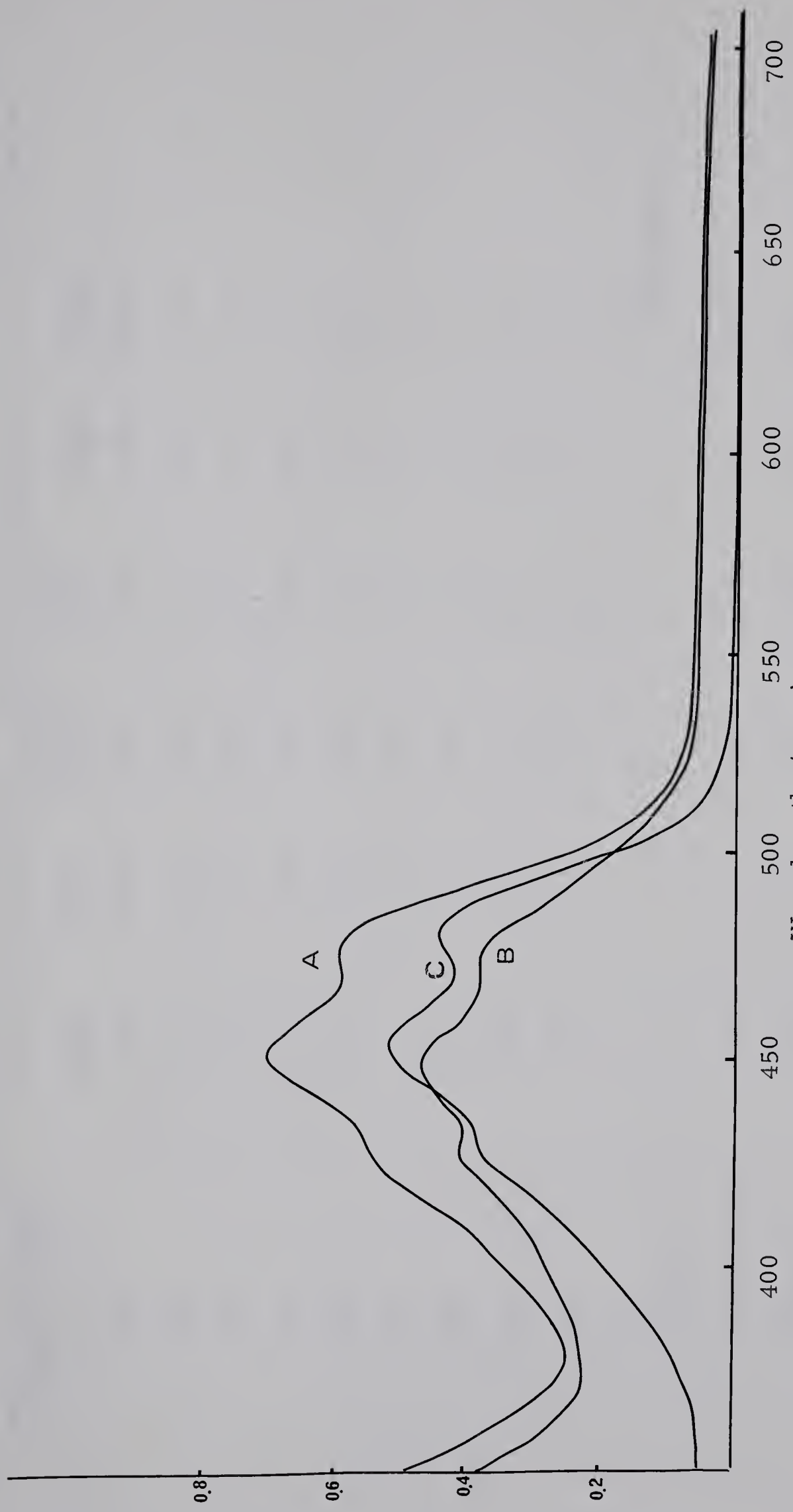


Figure 3. Visible Spectra of Milkfat in Hexane.
A. Fresh summer milkfat (3g/10 ml); B. Light-exposed milkfat, peroxide value 21 mEq /kg fat (3g/10 ml); C. pure β-carotene (22γ/10 ml)

Table 2. Effect on Milkfat Oxidation of Isolated Wavebands from Cool-White Fluorescent Lamp.

Peak wavelengths of filters (nm)	Intensity of oxidation (peroxide value, mEq/kg fat) at various exposed intervals (days)					
	1 day	2 days	4 days	6 days	8 days	10 days
410	0.28	0.30	0.67	1.04	1.60	2.08
443	0.30	0.42	0.83	1.14	2.00	2.10
476	0.22	0.14	0.54	0.86	1.12	1.17
510	0.25	0.25	0.67	0.64	0.90	1.22
543	0.35	0.42	1.00	1.20	1.76	2.03
576	0.42	0.38	1.56	1.75	3.53	4.40
610	0.15	0.14	1.17	1.32	2.25	2.25
643	0.08	0.14	0.12	0.33	0.63	1.00
676	0.05	0.14	0.42	0.74	0.83	1.00
710	-	-	-	-	-	- (not detectable)
Control	-	-	-	-	-	-
Without filter	3.50	6.45	13.5	21.9	46.2	50.8
360 (UV)	0.42	0.45	1.20	1.50	2.30	2.60
400	1.25	2.10	3.38	4.58	10.80	11.0
800 (IR)	-	-	-	-	-	-

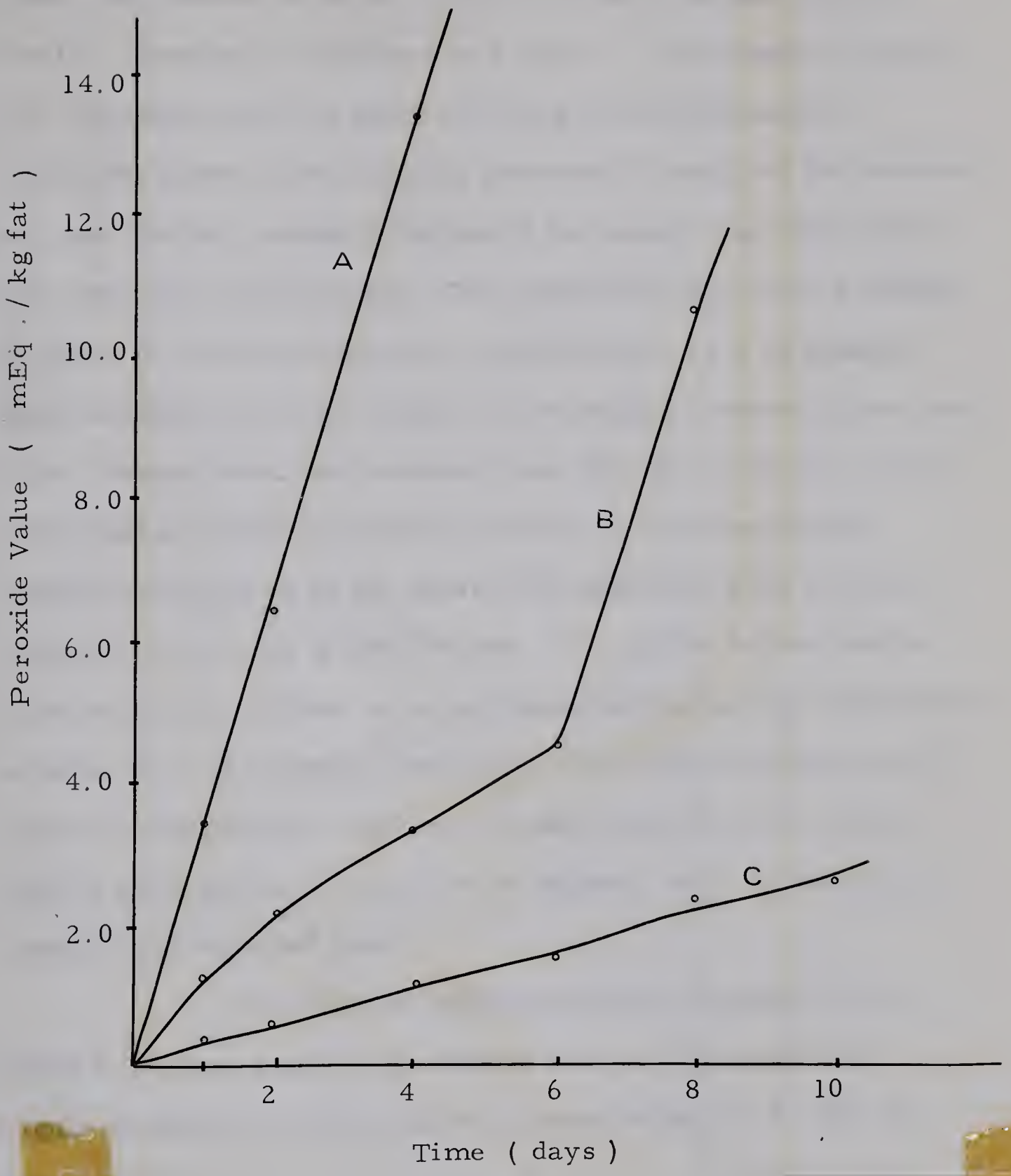


Figure 4. Effect of different amounts and wavelengths of light on the rate of milkfat oxidation
A. Milkfat exposed to the whole spectrum of Cool-White Fluorescent Lamp.
B. Milkfat exposed to waveband 400 nm (350-520 nm) .
C. Milkfat exposed to waveband 360 nm (310-410 nm).

The spectral energy distribution curve of the Cool-White Fluorescent Lamp as reported by the Canadian General Electric Company is indicated in Figure 5. The smooth, broken line represents only the light resulting from phosphorous excitation. Some visible light is generated directly by the mercury arc, and the bars added to the top of the broken line show where this energy is concentrated. The absorption spectrum of milkfat is shown in the same figure for comparision. As it is apparent from this figure that the range of wavelengths emitted by the Cool-White Fluorescent Lamp extends from 300 nm to 750 nm. Shorter wavelengths of light are either absorbed by the phosphorous coating materials or by the glass bulb. Maximum light energy is centered in the area of 560-590 nm, i.e., in the yellow portion of the visible spectrum. A second maximum in energy distribution is in the area of carotene absorption. The cross-hatched area in Figure 5 indicates the region in the spectrum in which emitted light is absorbed by the carotene in milkfat, but it is not proportional to the absorbed power.

The effect of milkfat oxidation of isolated wave-bands in various parts of the visible spectrum is shown in Figure 6 where peroxide values measured after 6-, 8- and 10-day exposures are plotted against the peak wavelengths of the filters used. It is noted that the area where carotene absorbs (400-550 nm) showed an increase in peroxide values. The

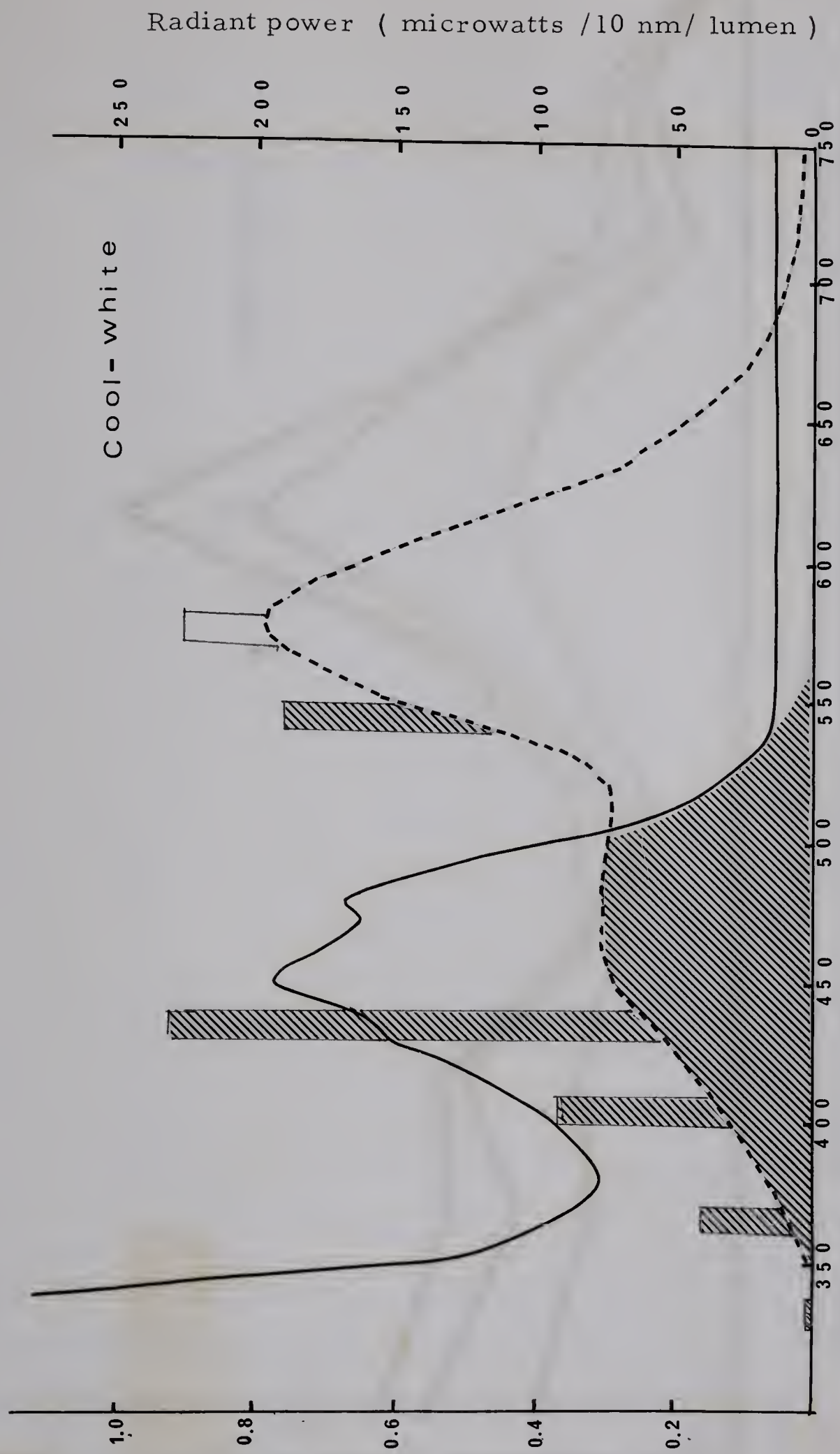
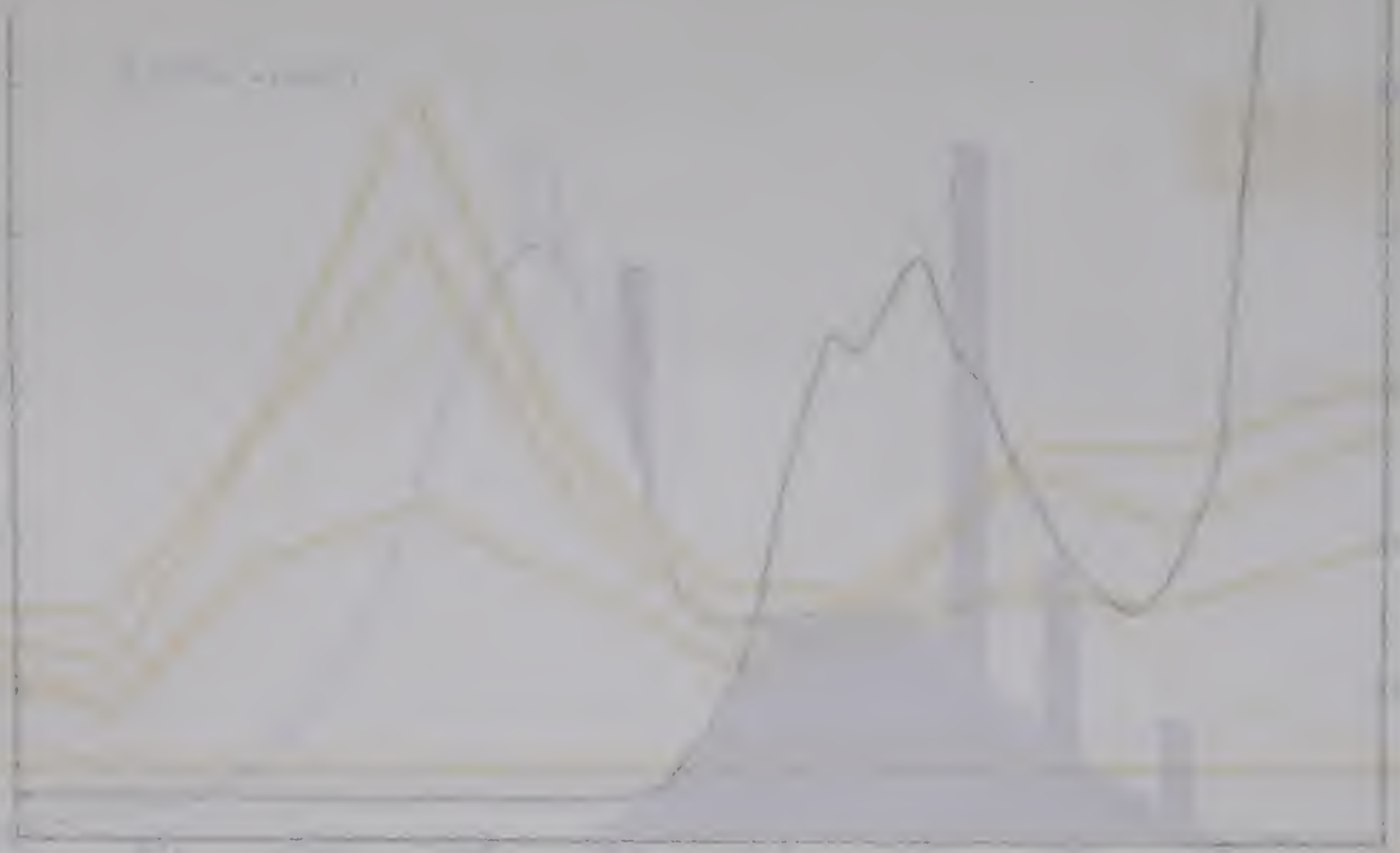


Figure 5. Emission spectrum of Cool-White Fluorescent Lamp with absorption spectrum of milkfat shown for comparision. Cross-hatched area indicates region in which emitted light is absorbed by carotene, but is not proportional to absorbed power. (Emission spectrum provided by the Canadian General Electric Co.)



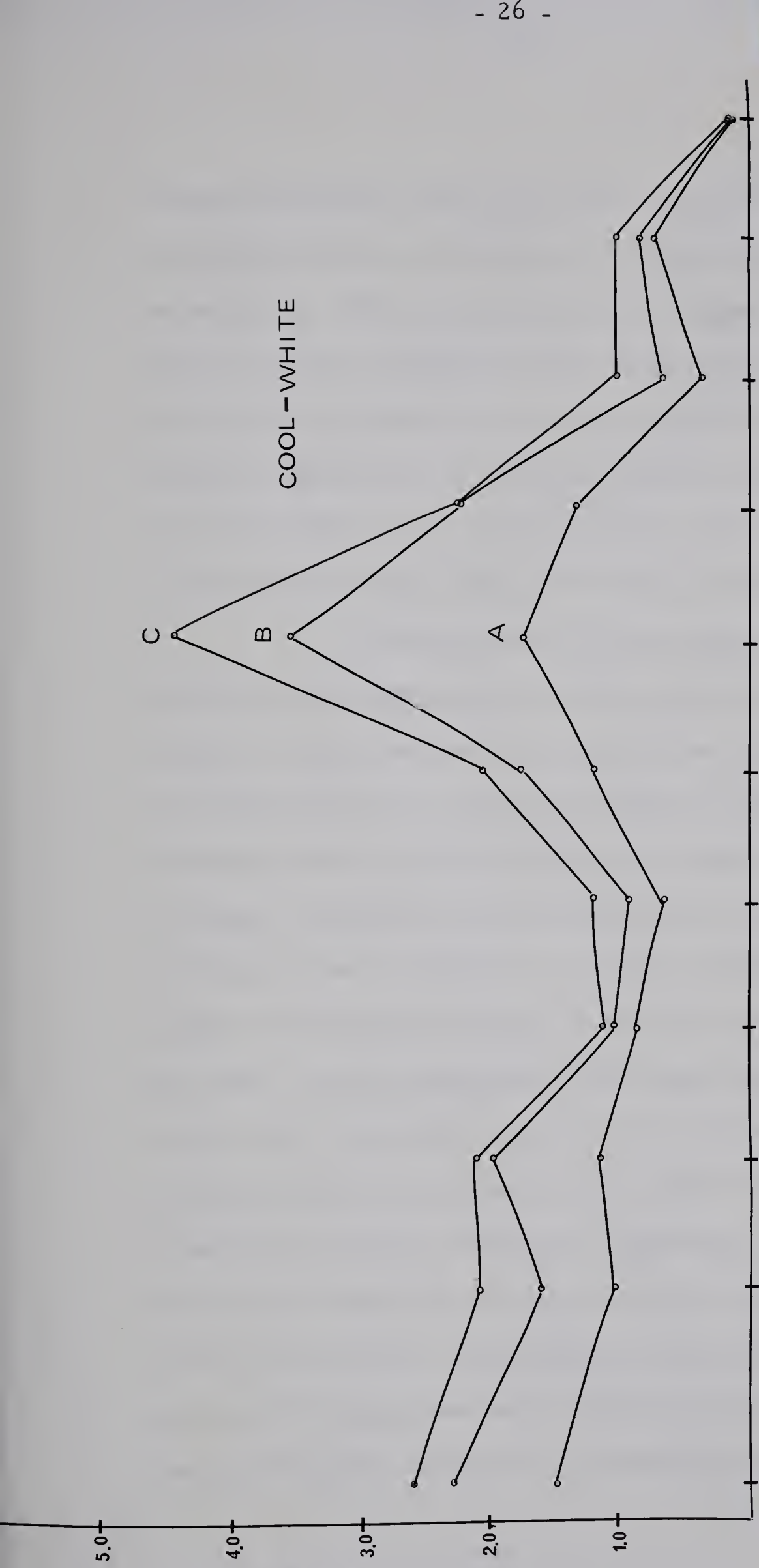


Figure 6. Effect on milkfat oxidation of isolated wavebands from Cool-White Fluorescent Lamp.
A. Milkfat exposed for 6 days.
B. Milkfat exposed for 8 days
C. Milkfat exposed for 10 days.

extent of oxidation was least at the red end of the spectrum. Of particular interest is the intense oxidation at 576 nm peak wavelength, which corresponds to the region of the spectrum where maximum light energy is most concentrated. Oxidation in the near ultraviolet range was pronounced but not to the same extent as that observed with 576 nm where no chromophore was detected. The pattern of oxidation was quite characteristic as shown by the similar shape of the three curves.

Thermocouple measurements of the energy transmitted by each waveband from four types of lamps is shown in Figure 7 where the detected energy in microwatts x 10^{-2} is plotted against the peak wavelengths of filters. The curve obtained with Cool-White Fluorescent Lamp showed resemblance in shape, except the near ultraviolet end, to the pattern of oxidation shown in Figure 6. The filter with 576 nm peak wavelength transmitted the highest detected energy and brought about the most intense oxidation in the samples exposed to this waveband. Other wavebands in the visible spectrum showed a similar trend, the area where more light energy was transmitted showed more intense oxidation; suggesting a possible relationship between incident light energy and extent of fat oxidation. Deviation from such observed relationship is noted in the near ultraviolet portion of the spectrum where the small value of detected light energy did not account for the pronounced oxidation observed.

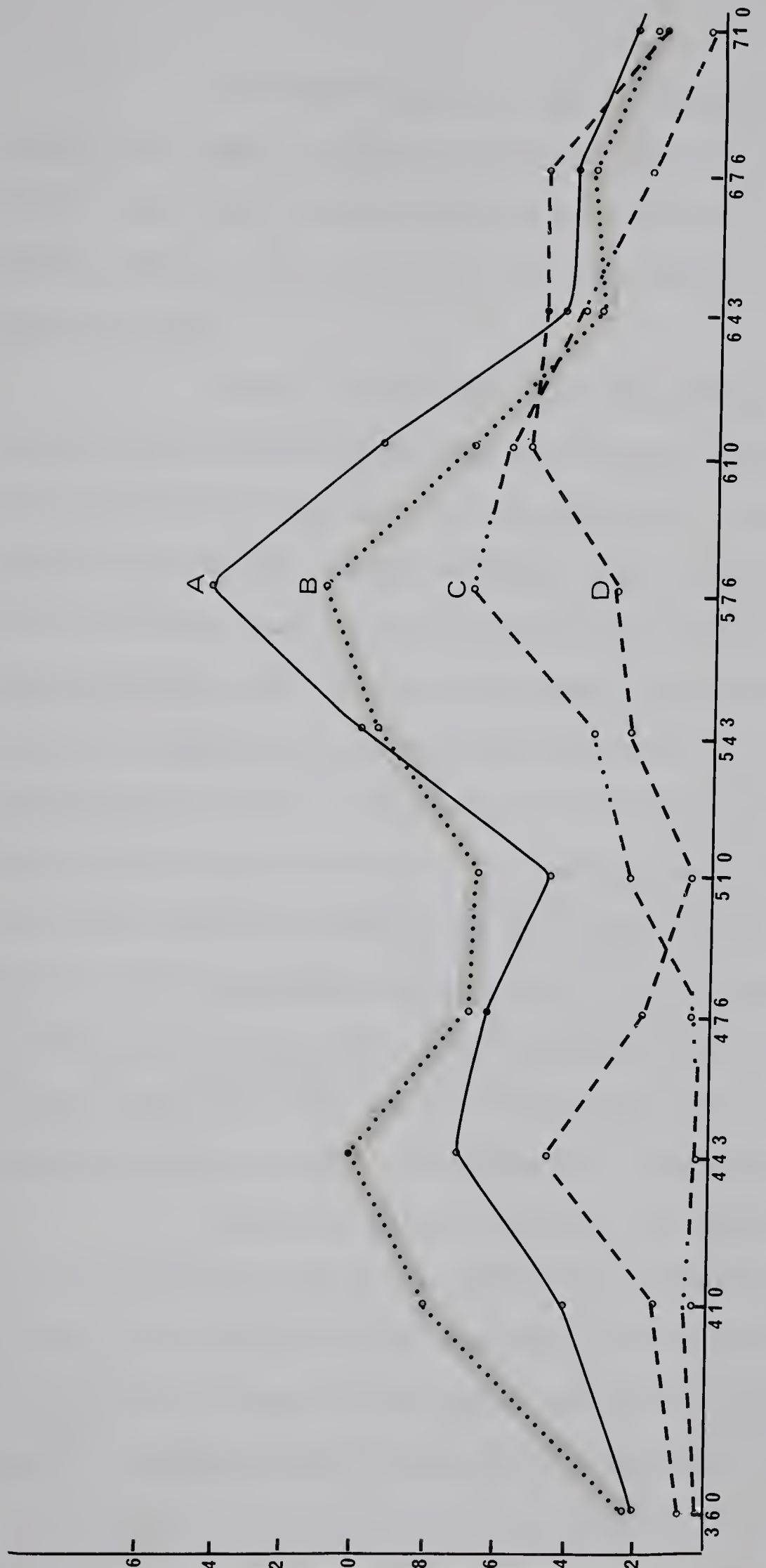


Figure 7. Relative intensity of transmitted ligh
A. Cool-White ; B. Daylight ;
Lamps.

The spectral energy distribution curve of Daylight Fluorescent Lamp is shown in Figure 8. Like the Cool-White Fluorescent Lamp, spectral energy maximum is centered in the yellow portion of the spectrum, a second maximum appears in the blue region.

Figure 9 shows the effect on milkfat oxidation of isolated wavebands from Daylight Fluorescent Lamp, where the extent of oxidation, measured in peroxide value, is plotted against the peak wavelengths of filters. Here, again, the 576 nm waveband which transmitted the most intense spectral energy emitted from Daylight Fluorescent Lamp, caused the samples exposed to this waveband to oxidize extensively. The pattern of oxidation was similar to that observed with the Cool-White Fluorescent Lamp except there was comparatively more oxidation in samples exposed to light in the blue region of the spectrum. Results with transmitted energy measurements (Figure 7) indicated a curve of similar shape except the near ultraviolet portion, which gave low energy readings but caused milkfat exposed to this waveband to be oxidized to a noticeble extent.

Figure 10 shows the energy distribution curve of the Pink Fluorescent Lamp. Unlike the previous two types of lamps, the spectral energy maximum of Pink Fluorescent Lamp locates at longer wavelengths; namely 610-620 nm. However, scattered in the blue and violet region are high energy

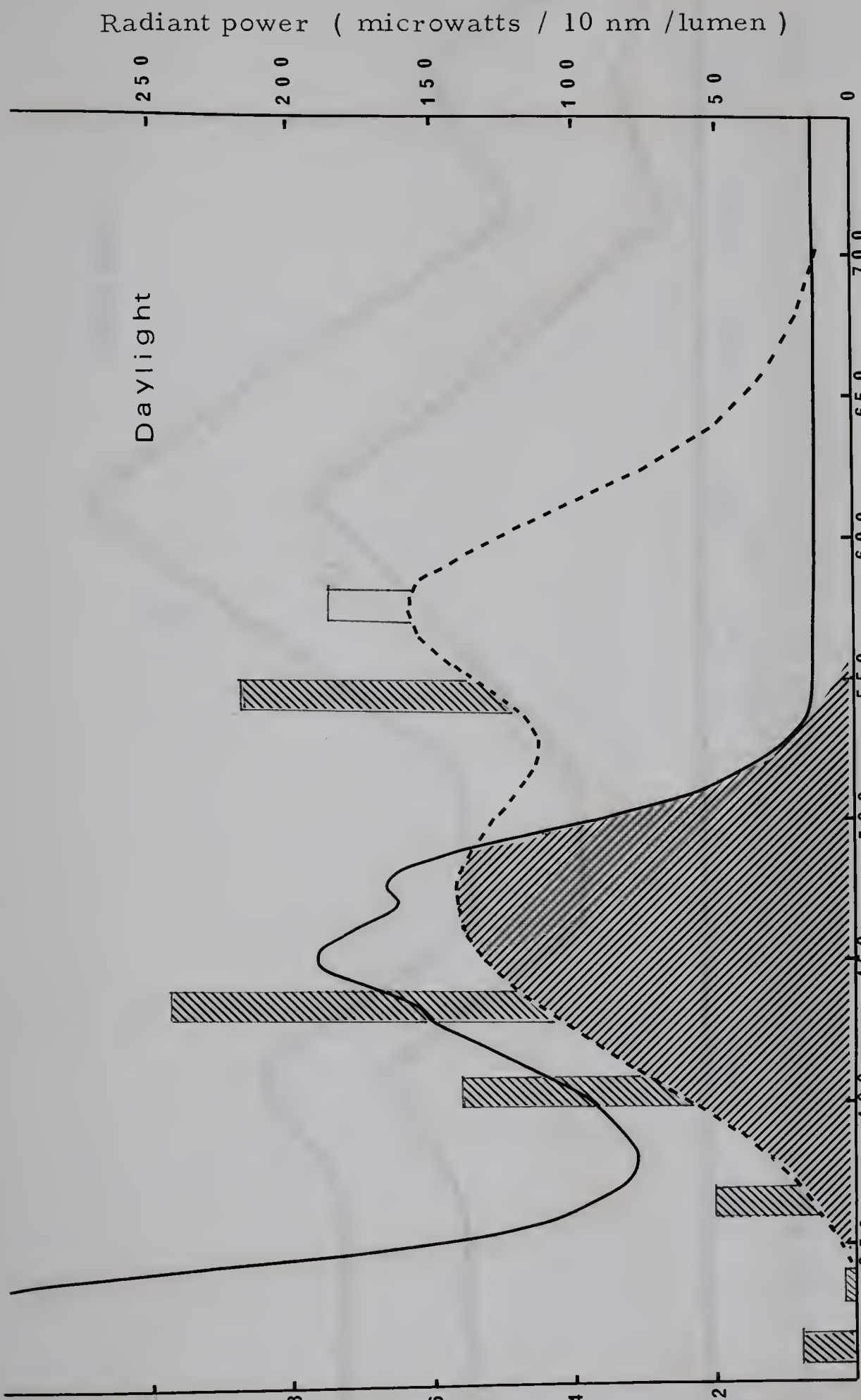
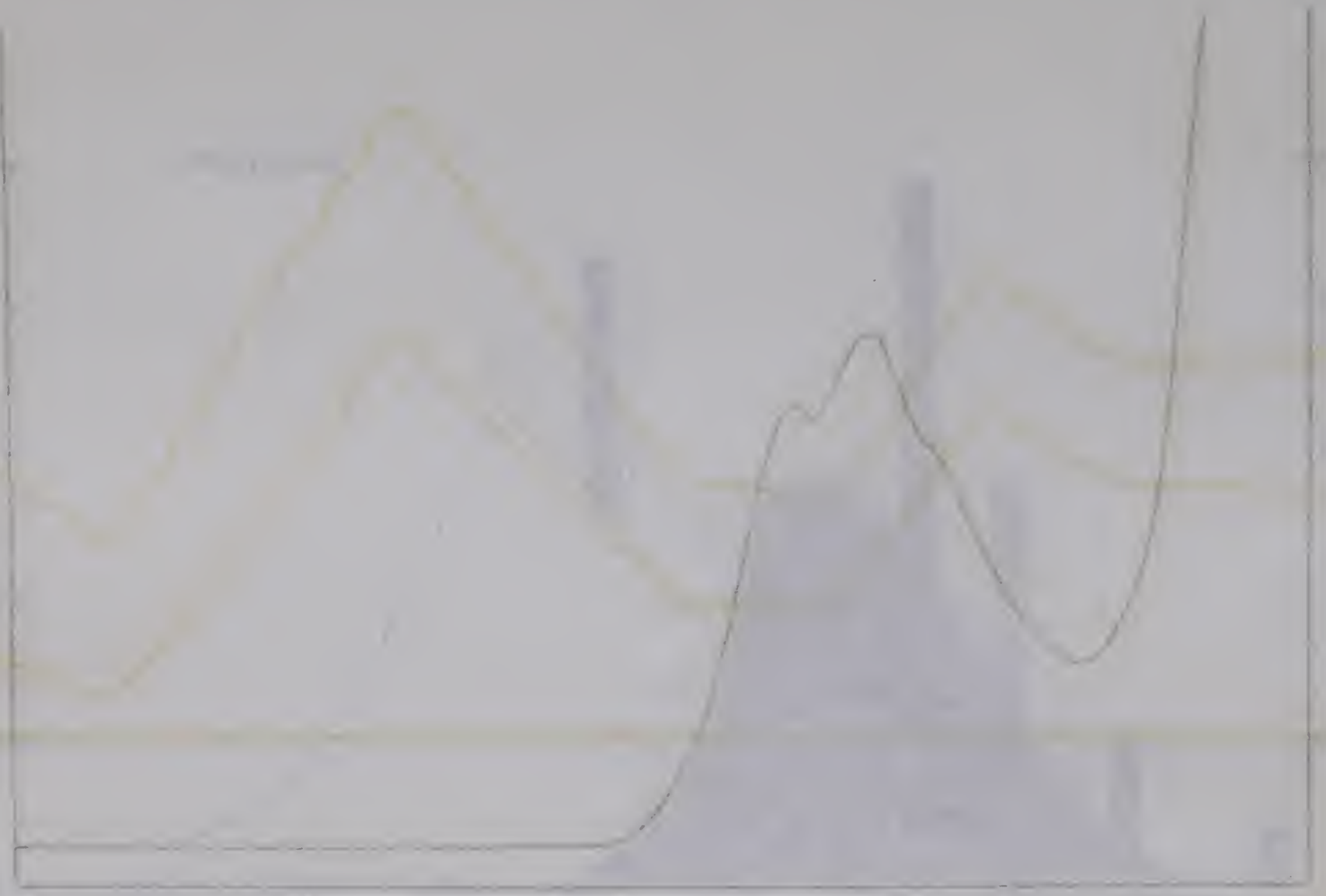


Figure 8. Emission spectrum of Daylight Fluorescent Lamp with absorption spectrum of milkfat shown for comparision. Cross-hatched area indicates region in which emitted light is absorbed by milkfat, but is not proportional to absorbed power. (Emission spectrum provided by the Canadian General Electric Co.)



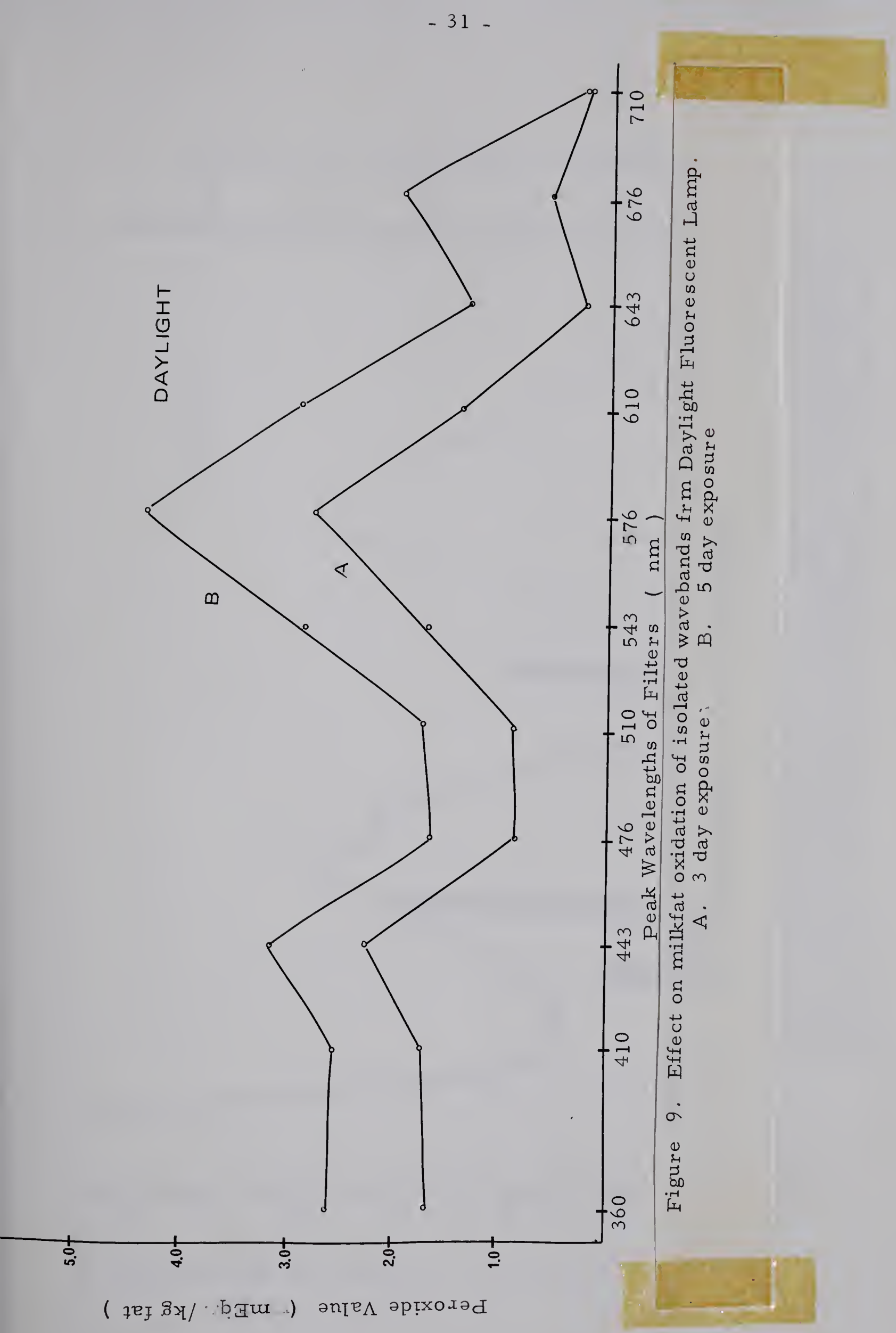


Figure 9. Effect on milkfat oxidation of isolated wavebands frm Daylight Fluorescent Lamp.
A. 3 day exposure B. 5 day exposure

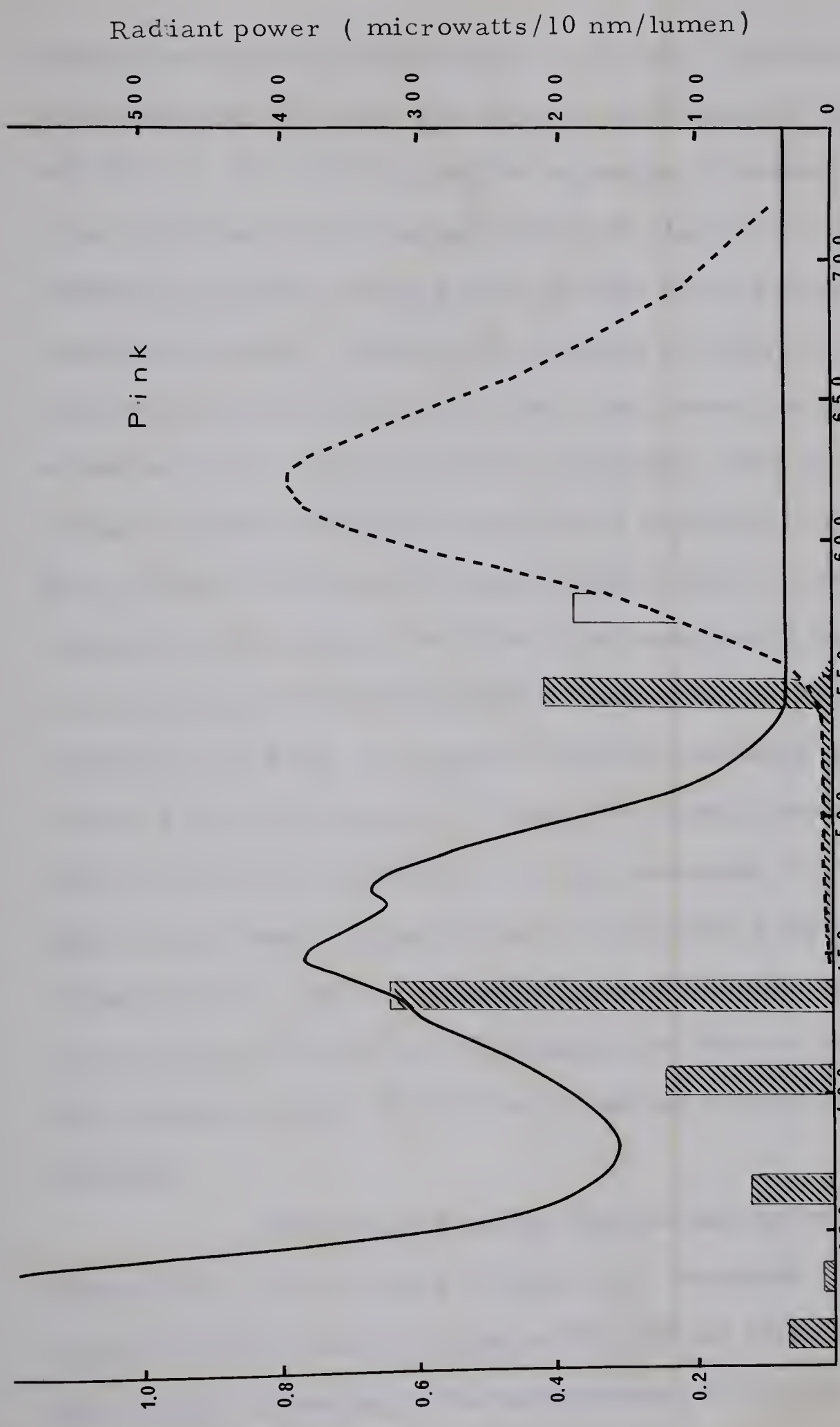
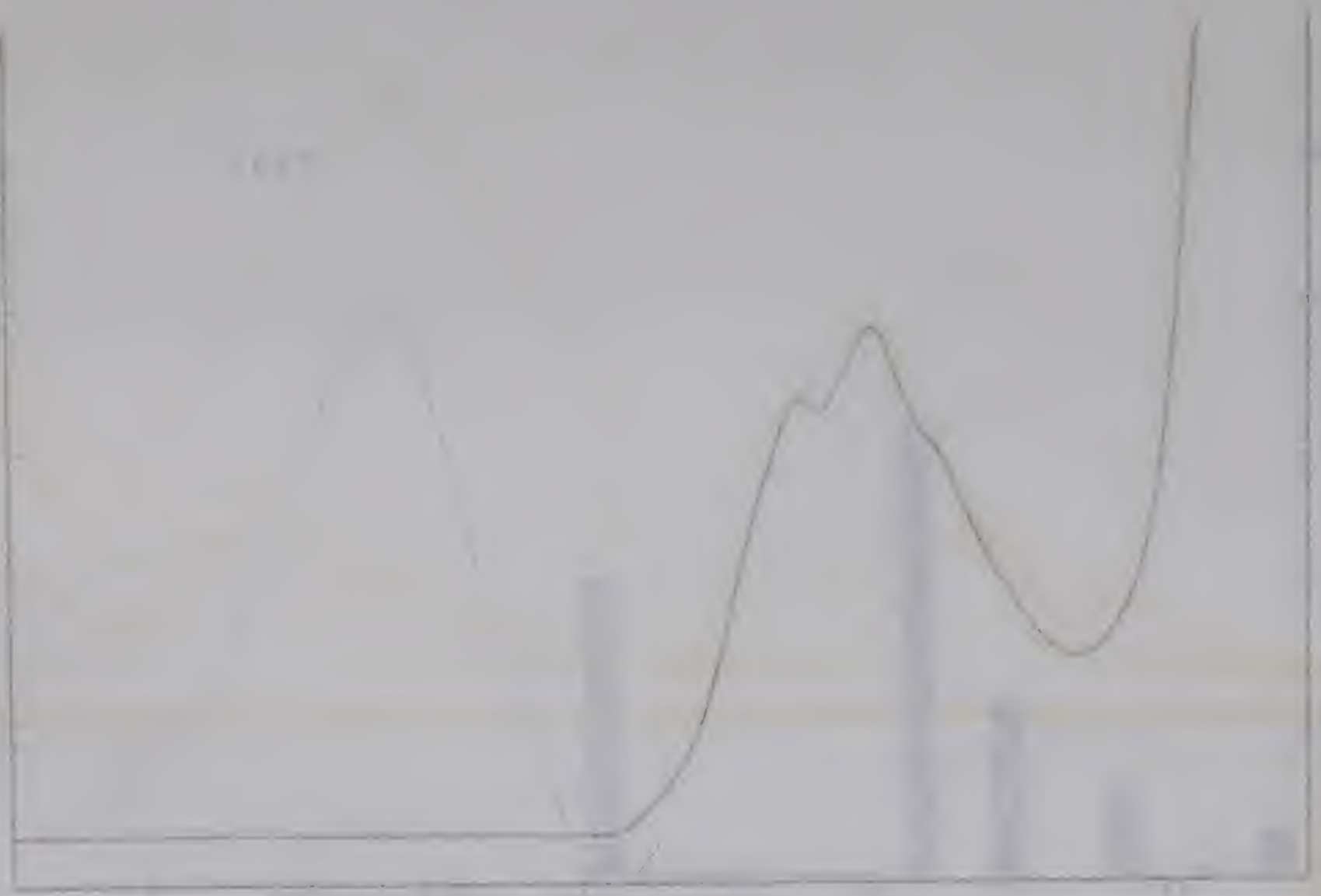


Figure 10. Emission spectrum of Pink Fluorescent Lamp with absorption spectrum of milkfat shown for comparision. Cross-hatched area indicates region in which emitted light is absorbed by carotene, but is not proportional to absorbed power. (Emission spectra provided by the Canadian General Electric Co.)



bands generated from the mercuric arc lines. There are, relatively speaking, low spectral energies distributed in the area of 450-540 nm. The effect on milkfat oxidation of isolated wavebands from Pink Fluorescent Lamp is shown in Figure 11. Intense oxidation of milkfat occured in the orange and red parts of the spectrum although, matching the mercury arc lines in the emission spectrum of Pink Fluorescent Lamp, there was also appreciable oxidation in the region of carotene absorption. The most intensely oxidized samples being those exposed to waveband 676 nm, samples exposed to 610 nm were more oxidized than any other milkfat exposed to other parts of the whole spectrum except those under the irradiation of 610 nm waveband. Instead of an oxidation maximum at 610 nm, as expected from the emission spectrum of Pink Fluorescent Lamp in Figure 9, oxidation maximum was observed under the radiation of 676 nm waveband. Transmitted light energy measurements(Figure 7) indicated a similar curve comparable to Figure 10 except the peak at 676 nm . Region of the emission spectrum of the lamp where there is relatively little radiant energy (450-540 nm) resulted in very little oxidation.

The spectral energy distribution curve of Gold Fluorescent Lamp is shown in Figure 12. Maximum spectral energy is centered in the region of 500-700 nm with a peak at 580-590 nm. According to the manufacturer (Canadian General

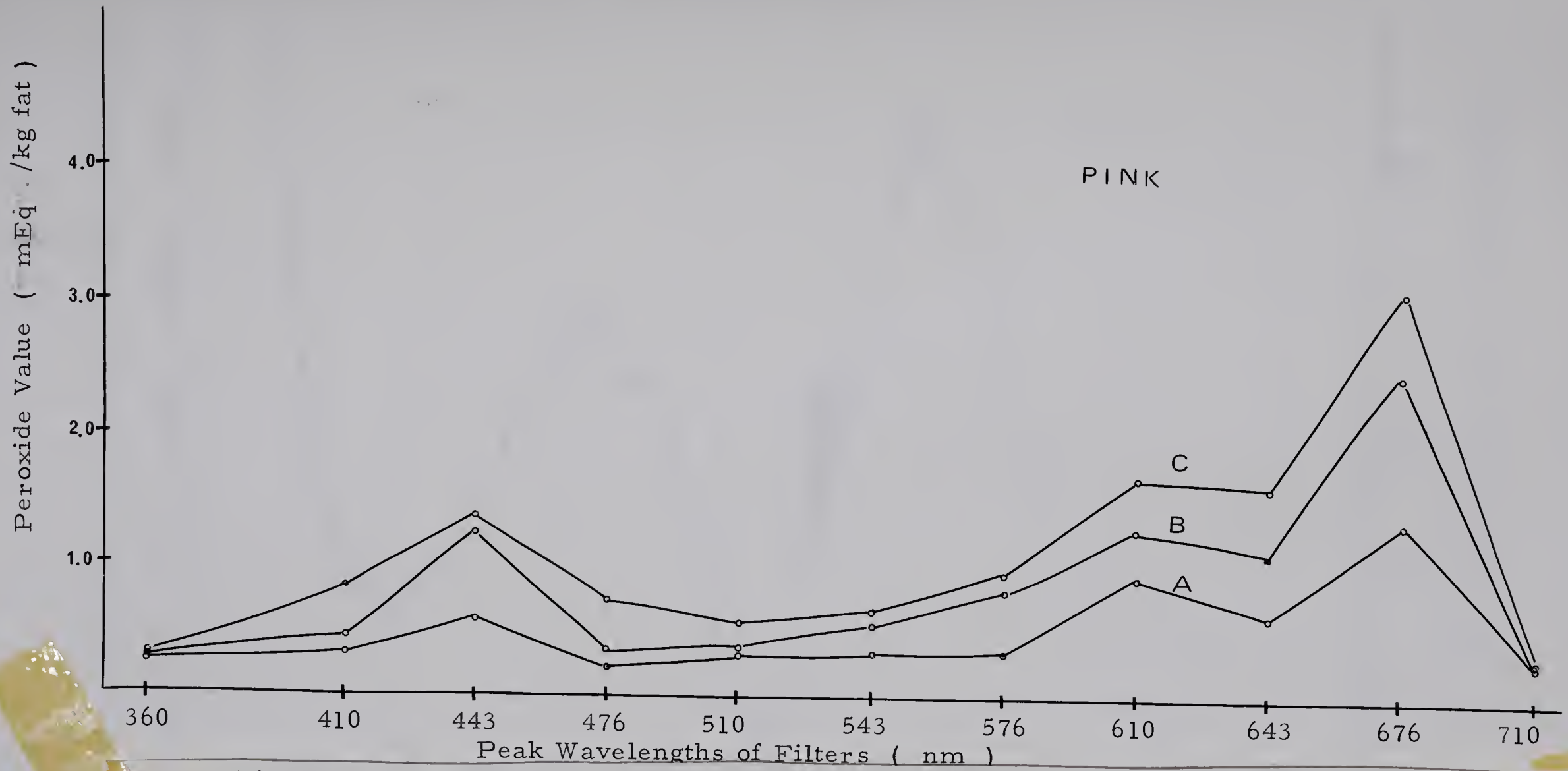


Figure 11. Effect on milkfat oxidation of isolated wavebands from Pink Fluorescent Lamp.
A. 5 day exposure;
B. 8 day exposure;
C. 10 day exposure.

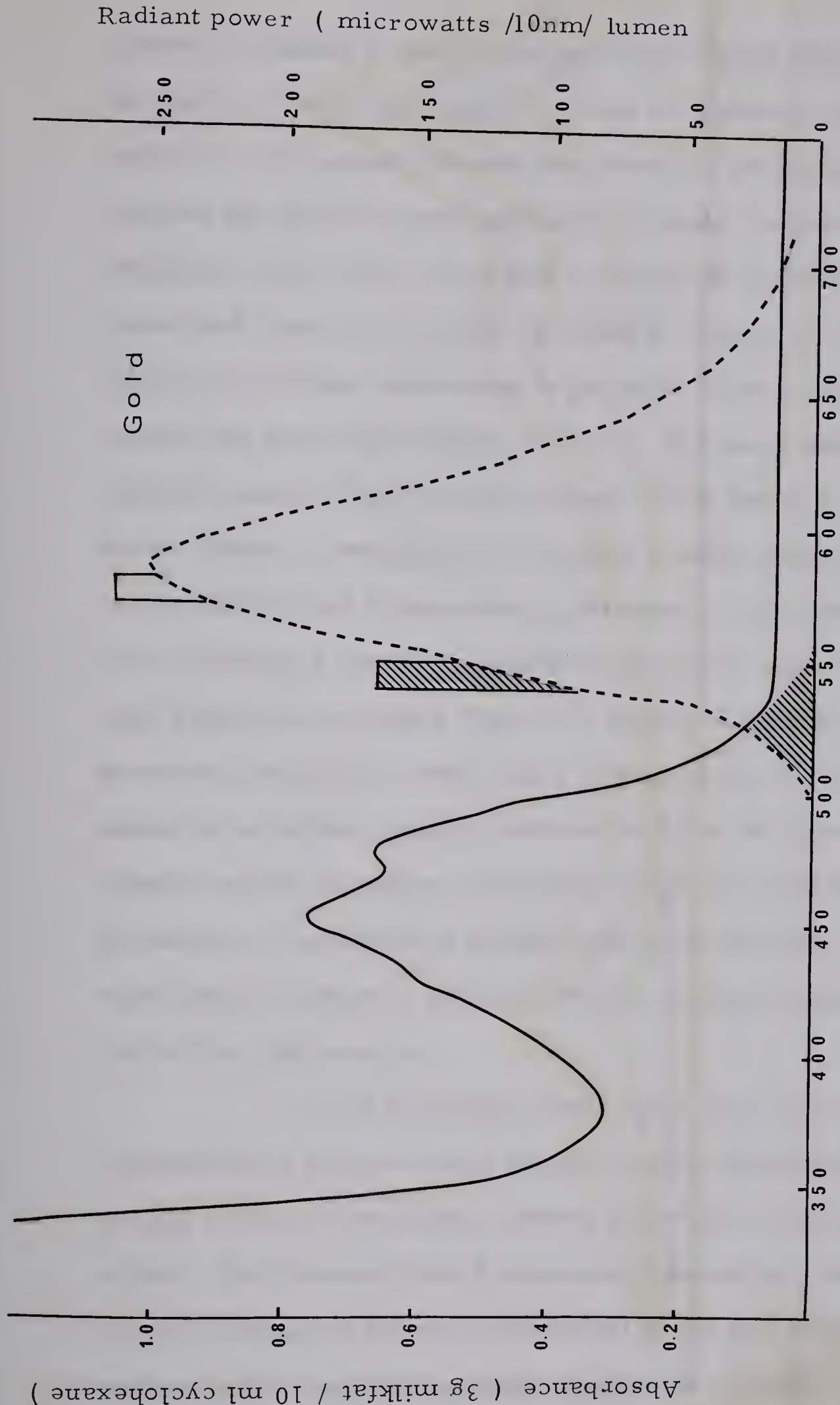
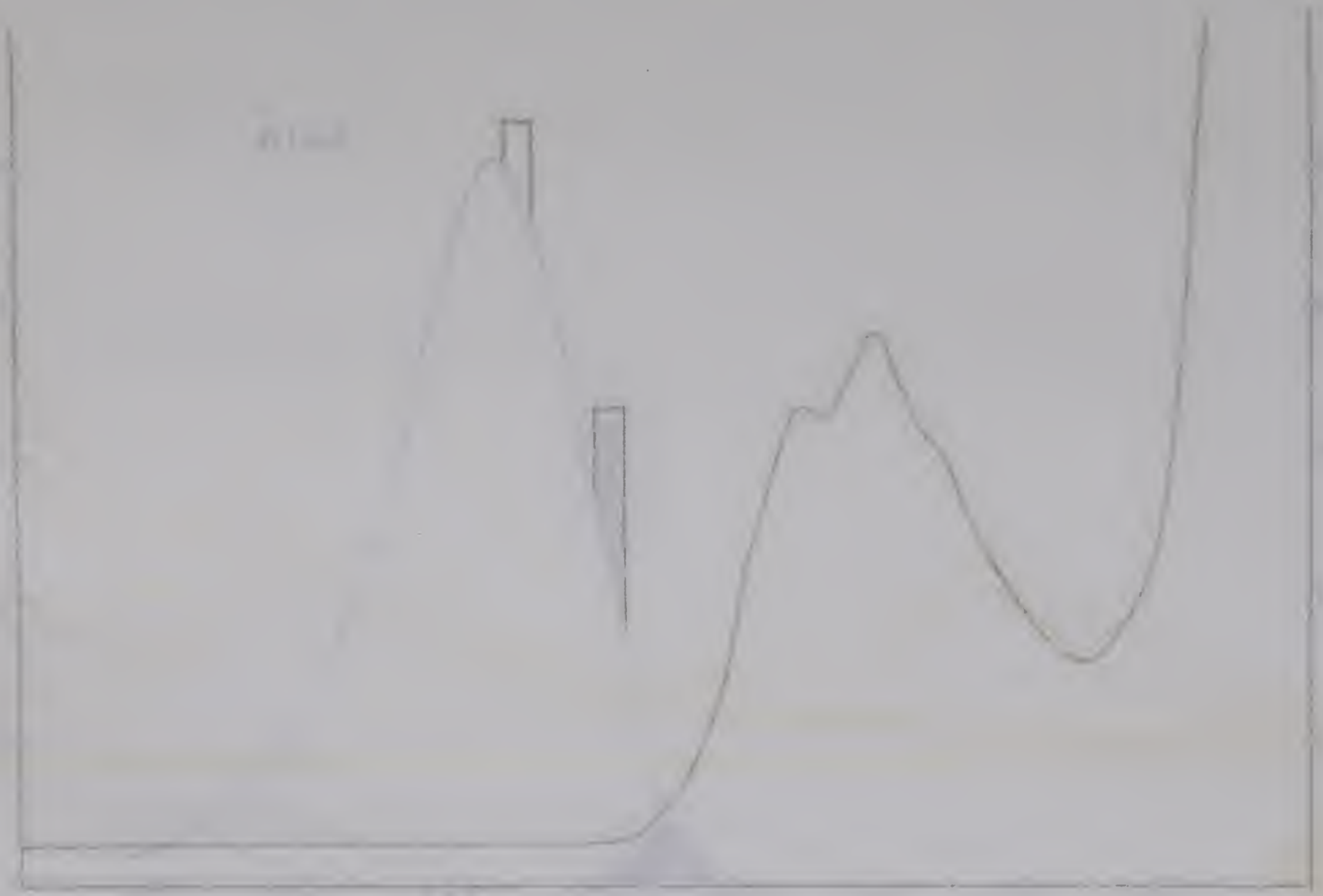


Figure 12. Emission spectrum of Gold Fluorescent Lamp with absorption spectrum of milkfat shown for comparision. Cross-hatched area indicates region in which emitted light is absorbed by carotene, but is not proportional to absorbed power. (Emission spectrum provided by the Canadian General Electric Co.)



Electric Company), there is no spectral energy distributed in the shorter wavelength region because the colored coatings inside the bulb absorb not only the directly radiated mercury but also the shorter wavelength energy bands generated by the phosphors. The effect on milkfat oxidation of isolated wavebands from Gold Fluorescent Lamp is shown in Figure 13, where the extent of oxidation, expressed in peroxide value, is again plotted against the peak wavelengths of filters. The most intensely oxidized samples are those exposed to 576 and 610 nm wavebands, which correspond to the region of most intense spectral energy of the Gold Fluorescent Light source. The almost identical curve obtained from the transmitted energy measurement with the Gold Fluorescent Lamp (Figure 7) further substantiated the previous findings with other types of fluorescent lamps in that oxidation of milkfat could be induced to occur by light of wavelengths outside the major absorption range of carotene, and that the pattern of oxidation of milkfat under the influence of isolated wavebands of light was altered with the spectral energy distribution of the light source.

In all the lamps used, there was little energy transmitted at the red end of the spectrum and milkfat exposed to light of these wavelengths showed no increase in peroxide values. The Pink and Gold Fluorescent Lamps gave very low energy readings in the near ultraviolet range and milkfat samples exposed to this radiation showed no observable oxidation in ten-day exposures.

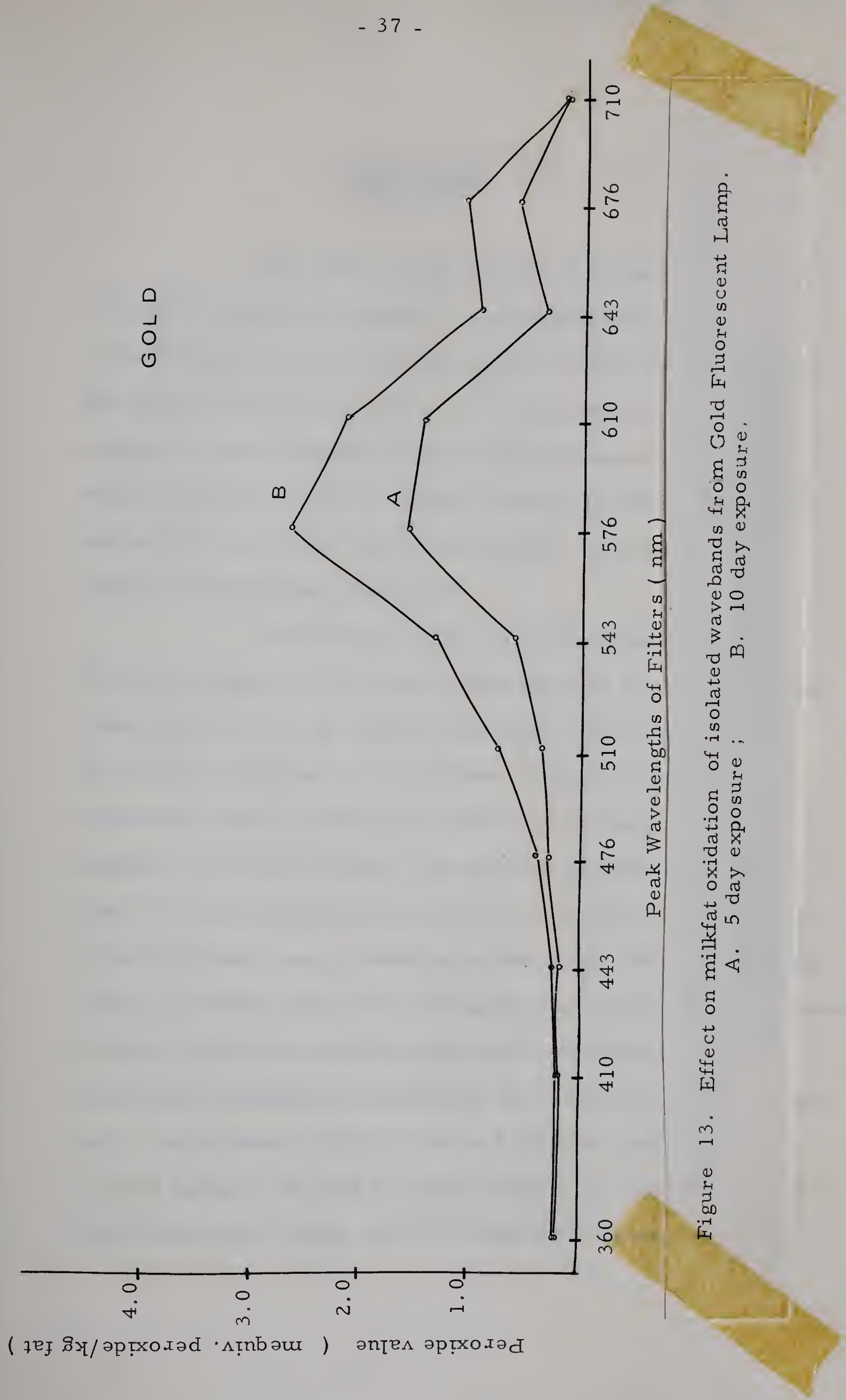
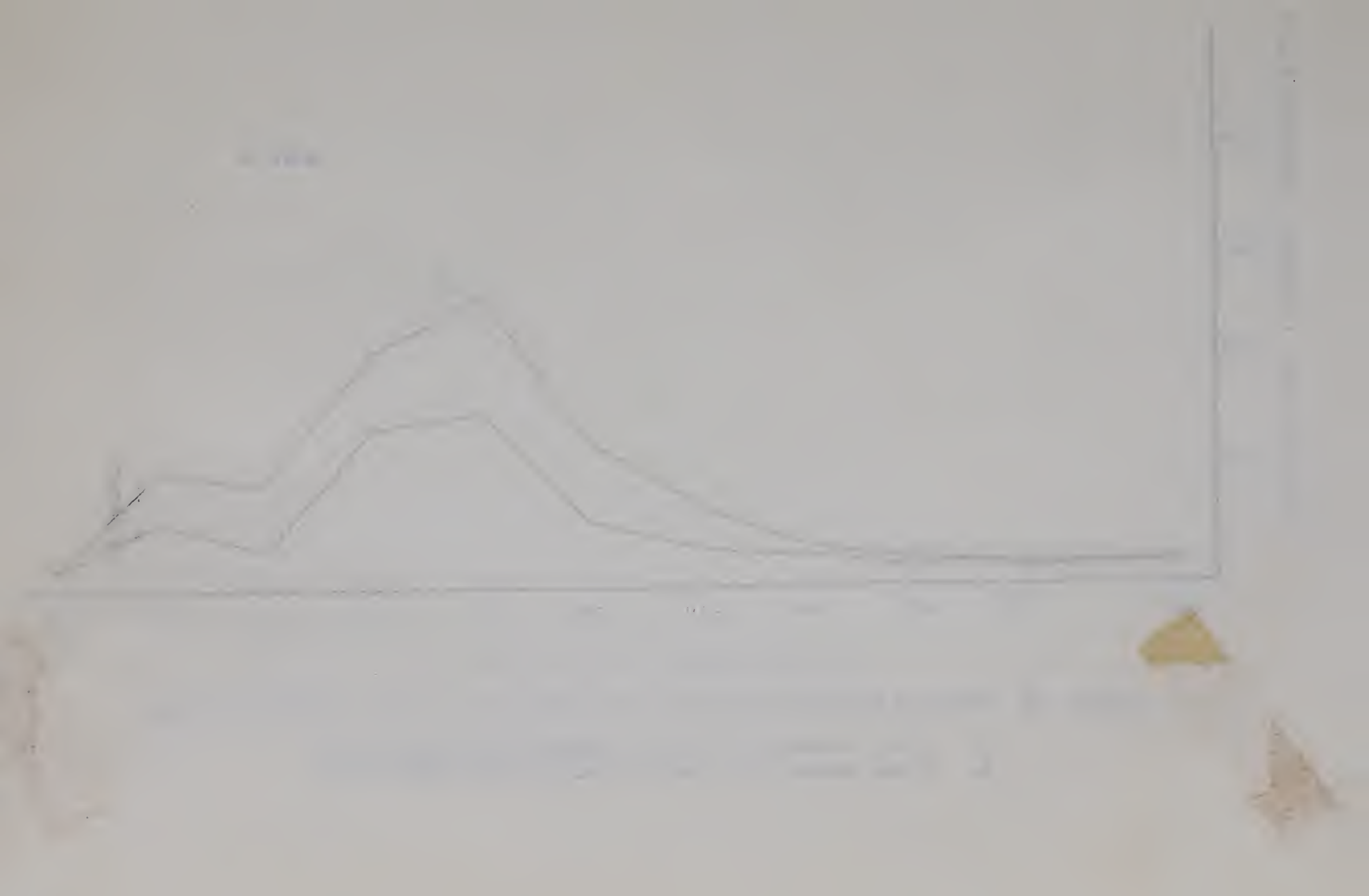


Figure 13. Effect on milkfat oxidation of isolated wavebands from Gold Fluorescent Lamp.
A. 5 day exposure ;
B. 10 day exposure.



DISCUSSION

The effect of visible light in promoting oxidation in milkfat was quite unexpected, since photochemical reactions are understood to be wavelength specific and to occur only when the system under investigation absorbs photons of certain energies. It was therefore interesting to observe the extent to which oxidation occured in milkfat exposed to light of the yellow and orange part of the spectrum where the reacting substrate exhibited insignificant absorption.

From the spectral energy distribution curves of the four types of fluorescent lamps used, it was observed that over half of the energy output of the light sources was outside the major absorption area of milkfat. Except for the small discrepancy observed with Pink Fluorescent Lamp, milkfat samples in all cases showed the greatest amounts of oxidation when they were exposed to parts of the spectrum corresponding to the maximum energy emission of the lamps. When the peroxide values of milkfat exposed for the same time to different wavebands of visible light were plotted against the transmitted energy, statistically significant relationship was found with all the lamps used. Correlation coefficients were 0.90 with Cool-White Fluorescent Lamp, 0.85 with Daylight Fluorescent Lamp, 0.82 with Pink Fluorescent Lamp, and 0.95 with Gold Fluorescent Lamp.

The method used to detect light energy measured only part of the transmitted energy and therefore the accuracy of the reported values is relative rather than absolute. A better, non-selective instrument, capable of measuring the total number of photons absorbed, would be required to permit calculation of quantum yields.

In the set of visible light filters, the one with its transmission maximum at 676 nm had the widest transmission band (Table 1) and undoubtedly this filter did transmit more light in the region concerned. This may account for the rather intense oxidation observed in milkfat samples exposed to 676 nm waveband isolated from all four types of fluorescent lamps used and perhaps explains the observed oxidation maximum where the Pink Fluorescent Lamp was used as the light source. Transmission characteristics of light filters should be taken into consideration particularly when no other information is available on the total amount of light actually transmitted or absorbed by the system under investigation. This variation in transmission characteristics could also account for the extensive oxidation of milkfat samples exposed to light of the near ultraviolet range isolated from Cool-White and Daylight Fluorescent Lamps since the Corning glass color filter (Table 1) used to isolate the near ultraviolet light band possessed an effective bandwidth 2 1/2 times the width of any of the visible light interference filters used. In addition, the strong absorption of milkfat in the

near ultraviolet range could explain, at least in part, the extensive oxidation observed in that portion of the spectrum.

Lea in 1931 reported that the extent of fat rancidity varied with the intensity of either sunlight or light from a gas-filled lamp. Similar relationship was also observed by other workers (Greenbank, 1941 ; Morgan, 1935.).

The mode of action of light in fat oxidation is not known although it has been postulated that light may function as an energy source which initiates free radicals. The pattern of oxidation observed in this investigation suggests that some component or components, the presence of which could not be detected spectrophotometrically, absorbs visible light energy and transfer this absorbed energy to the reacting substrate.

Carotene absorption may also account for part of the oxidation observed in the shorter wavelength region. However, because extensive oxidation occured at longer wavelengths as well, the effect of carotene absorption must not be overemphasized.

Lundberg (1967) stated that in his study of the peroxidation of methyl linoleate in the presence of chlorophyll and light of wavelength 660 nm at 37 ^{o}C, the oxidation proceeded extremely rapidly compared with autoxidation at the same temperature in the absence of chlorophyll. He stated that the photo-chlorophyll reaction involves mechanisms quite different from

those of ordinary autoxidation. The presence of chlorophyll could be detected spectrophotometrically. However, chlorophyll is not present in milkfat and it is not understood whether the oxidation of milkfat by visible light involves other unknown constituents.

Attention should be drawn to the fact that most work on fat oxidation has used liquid systems. Oxidation of solid fats might not involve the same mechanisms proposed in liquid oils. Since the physical state of the substrate fat in the experiments was solid, it is doubtful whether unabsorbed light does transmit through the samples, as in the case of the absorption spectrum of milkfat solution. But rather the light might penetrate the fat solid to a certain depth and be absorbed, reflected or fluoresced. It had been estimated that about 98 % of the light, of wavelengths less than the absorption threshold, which enters a typical crystal may be absorbed within a distnace of 10^{-6} cm, of about 100 atomic layers (Calvert and Pitts, 1966). There is always an uncertainty in the physical state of the reactants absorbed on solids. Oxidation of milkfat in the presence of light has been shown to occur mainly on the surface of the substrate fat. It is only when the oxidation has proceeded to a certain extent that oxidation products begins to accumulate in the lower layer of the fat (Pimentel, 1966 ; Gilchrist et al. 1968.). Thus, it is speculated that light of wavelengths outside the major absorption of milkfat might

produce a surface absorption effect. This absorbed energy might be dissipated as heat to the surroundings or might produce a thermal effect to excite the molecules of the reacting substrate and render them more susceptible to oxidation.

However, photooxidation in the solid phase is too complicated to afford any acceptible explanation. The actual process might bring into play a variety of factors which operate simutaneously and are not well understood at the present state of investigation.

SUMMARY

Milkfat oxidation is promoted by all visible wavebands except the 710 nm waveband isolated from four types of fluorescent light sources. Oxidation occured in the yellow, orange and red portion as well as the blue and violet range where milkfat exhibited major light absorption.

The influence on milkfat oxidation of isolated wavebands from Cool-White, Daylight, Pink and Gold Fluorescent Lamps varied in different parts of the visible spectrum with the amount of transmitted energy. The pattern of oxidation was consistent with the spectral energy distribution of the light source under consideration.

PART 2. OBSERVATIONS ON THE NATURE OF MILKFAT OXIDATION PROMOTED BY VISIBLE LIGHT.

The foregoing results have shown that milkfat oxidation is promoted by visible light outside the major absorption range of the observable chromophore in milkfat (carotene). This is difficult to explain from the photochemical standpoint, because only absorbed photons can participate in photochemical reactions. A logical explanation would be possible however if the existence could be demonstrated for heretofore undetected components which play a role in related photochemical reactions.

Accordingly, experiments were conducted to observe the pattern of oxidation in a number of treated milkfat samples exposed to isolated wavebands of light from the Cool-White Fluorescent Lamp.

Experimental Methods

Light source. The light source consists of two new Cool-White Fluorescent Lamp, F48T12CWHO, mounted 1 1/2 inches apart in high ouput type of fixture as previously described.

Addition of benzoyl peroxide. Milkfat was prepared in the usual manner by collecting the oil layer after

centrifuging melted butter at 45 °C. A calculated amount of reagent grade benzoyl peroxide was dissolved in a minimum amount of carbon tetrachloride (2 ml) and mixed with 130 g melted milkfat to give a mixture having a peroxide content of 2 mEq/kg fat. Equal portions (9 ml) of the mixture were transferred by pipet into aluminum holders, covered with filters and exposed to various periods of time under the fluorescent light as previously described. Milkfat without added benzoyl peroxide was exposed in the same fashion for comparision.

Removal and addition of carotene. Freshly activated charcoal powder (Darcoal, activated at 180 °C overnight) was mixed with melted milkfat in the ratio of 1 : 9 (w/w). The mixture was tempered in 45 °C water bath and filtered with suction through Whatman No. 42 filter paper in a Buchner funnel. The resulting de-pigmented sample resembled lard and had an $E^{1\%}_{1cm}$ value of less than 0.0016 at 450 nm, the absorption maximum of carotene in hexane. Visible spectra were recorded to observe the efficiency of carotene removal and the change in light-absorbing pattern of the treated milkfat.

β-carotene, dissolved in a minimum amount of petroleum ether, was mixed with a portion of the de-pigmented milkfat to bring the absorbance at 450 nm up to the original value ($E^{1\%}_{1cm}$ 0.0083 for this sample of winter milkfat).

A sample of the original milkfat, a portion of the de-pigmented milkfat and the de-pigmented milkfat with added carotene were exposed in usual fashion to narrow wavebands of light from Cool-White Fluorescent Lamp.

Carotene destruction under light. Several 9-ml portions of milkfat were pipetted into square aluminum holders and were exposed to the full spectrum of light from the Cool-White Fluorescent Lamp at a distance of four inches from the light source. Samples were taken at hourly intervals for both peroxide and carotene measurements. Carotene content was conveniently measured by the absorbance of the milkfat solution in hexane at 450nm.

Addition of phospholipids. Pure phosphatidyl ethanolamine isolated from egg yolks* and separated on a silicic acid column was obtained in the form of a solution in chloroform. 1 ml of this solution, containing 125 mg of phosphatidyl ethanolamine was mixed with 125 ml of tempered de-pigmented milkfat. Equal portions (9 ml) of this mixture were transferred to aluminum holders, covered with filters and exposed to Cool-White Fluorescent Lamp. The purity of the phosphatidyl

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* A generous gift from J.S. Chen, Department of Biochemistry, University of Alberta, Edmonton.

ethanolamine was checked by thin layer chromatography on silica gel G. The solvent system employed was $CHCl_3$: CH_3OH : NH_4OH : H_2O (60 : 35 : 2 : 3) . The phosphatidyl ethanolamine sample showed only one spot (detected by iodine vapor) with a R_f value of 0.81, identical with the R_f value of an authentic sample of phosphatidyl ethanolamine under the same conditions.

Milk phosphatides were extracted from butter serum using an alcohol-carbon tetrachloride mixture and repeated precipitation with acetone (Koops and Pette, 1956.). Thin layer chromatography gave tentative identification of phosphatidyl choline and phosphatidyl ethanolamine as constituents, and one unidentified component, having a R_f value of 0.30, was also found to be present.

One milliliter of the milk phosphatide extract, containing 143 mg of phosphatide residue, was mixed with 130 g melted de-pigmented milkfat. Equal portions (9 ml) were transferred to aluminum holders, covered with filters and exposed to Cool-White Fluorescent Lamp.

Iodine value determination. Iodine value determinations by the Wijs method (Cocks and Rede, 1966) were made on several samples to assess the effect of unsaturation on the milkfat oxidation pattern.

Oxidation of milkfat under light in a nitrogen atmosphere. Melted milkfat was de-aerated with pure nitrogen gas

for 20 minutes, and quickly transferred in 9-ml portions to aluminum holders which were inserted into the openings of the wooden box (p.13) and covered with filters. The whole box was placed in a glass cylinder, 8 inches in diameter and 18 1/2 inches long, having one sealed end and one open end with ground flange. A glass cover similar to a desiccator lid tightly sealed the open end. By means of stopcocks at either end of the cylinder, the unit was partially evacuated and then purged with nitrogen gas for one hour. Finally, with all stopcocks closed and the box of sample inside, the unit was placed at a distance of 9 inches from the light source and irradiated for 5 days during which the cylinder was purged with nitrogen for one hour each day. For purpose of comparision, milkfat samples were exposed to Cool-White Fluorescent Lamp in the normal way. At the end of this time, the extent of oxidation was measured in both sets of samples.

Rate of oxidation at 576 nm. Milkfat samples exposed to the 576 nm waveband were analyzed for peroxide content at various time intervals.

RESULTS

Addition of benzoyl peroxide to fresh milkfat in the calculated amount (2 mEq/kg fat) resulted in a value of 1.90 mEq/kg fat as detected by the iodometric procedure. This is because the iodometric procedure usually gave somewhat lower value due to reabsorption by the unsaturated lipids of the iodine liberated (Lea, 1962.). The control samples (benzoyl peroxide added and kept in the dark) indicated a small rise in peroxide value during the exposed period to 2.20 mEq/kg fat, suggesting the possible occurance of oxidation, even in the dark, at the level of added peroxide.

The pattern of oxidation in milkfat containing benzoyl peroxide is shown in Figure 14. Exposure times were 3 and 5 days for samples containing benzoyl peroxide and 5 days for samples without benzoyl peroxide. The samples containing benzoyl peroxide showed extensive oxidation when they were exposed to light of the near ultraviolet portion of the spectrum but only slight oxidation when exposed to any waveband in the visible range. The control samples without benzoyl peroxide showed the characteristic oxidation pattern and it is interesting that this pattern was not shown in the samples containing benzoyl peroxide. Most surprising, perhaps, the extent of oxidation in samples exposed to the 576 nm waveband was less for the sample

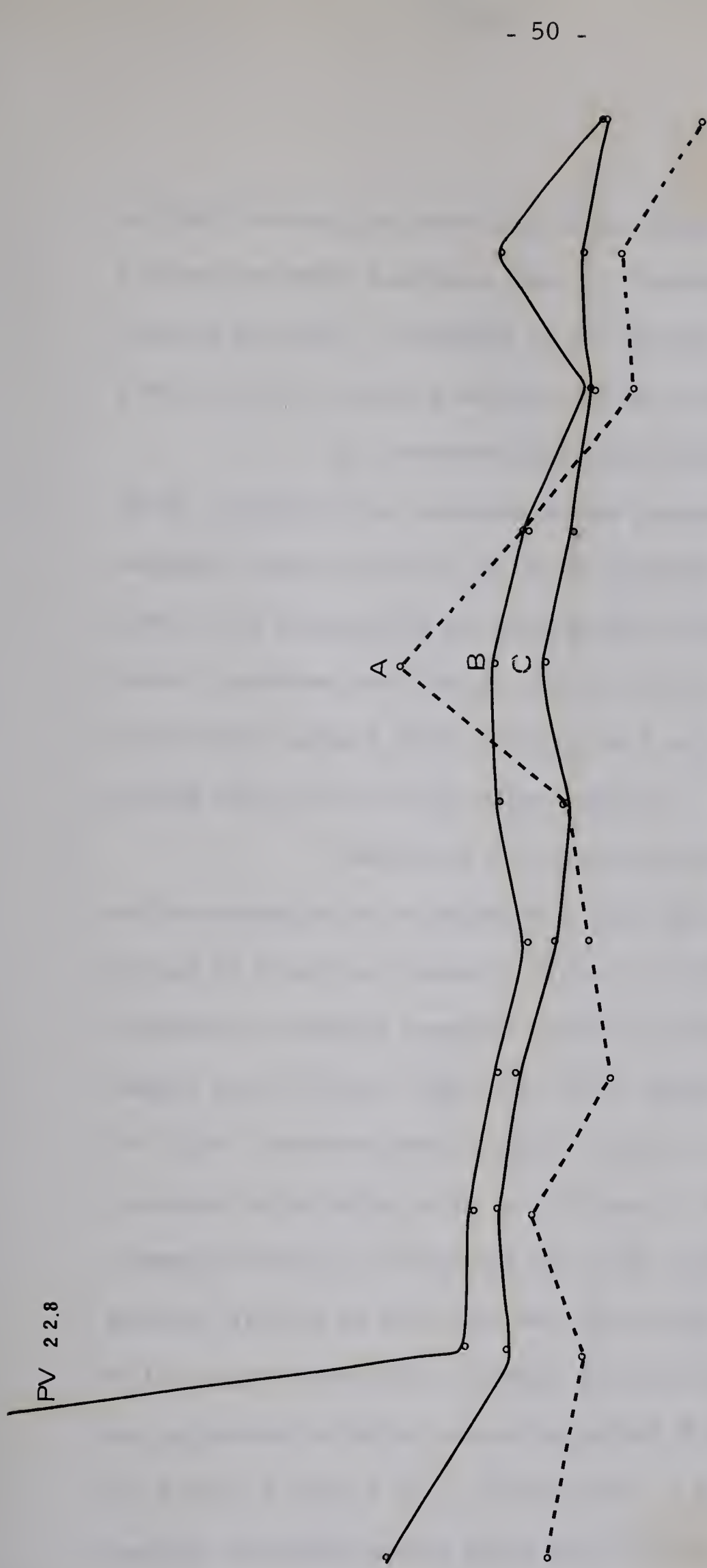

Figure 14. Effect of isolated wavebands of Cool-White Fluorescent Light on the oxidation of milkfat containing benzoyl peroxide.
A. Original milkfat exposed for 5 days.
B. Milkfat containing benzoyl peroxide, 5 day exposure.
C. Milkfat containing benzoyl peroxide, 3 day exposure.

to which benzoyl peroxide had been added than for that to which no benzoyl peroxide had been added. Therefore, at the level of added benzoyl peroxide, oxidation in the visible region under Cool-White Fluorescent Lamp was not related to incident light intensity.

In activated charcoal-treated milkfat, almost all of the carotene was removed in the process as shown by the absorption characteristics of the de-pigmented sample (Figure 15). There was essentially no absorption in the region of 400-530 nm where carotene absorption is normally observed. A slight increase in peroxide value (0.36 mEq/kg fat) occured in the control sample during the process of de-pigmentation.

The effect of isolated wavebands of visible light on the oxidation of de-pigmented milk fat after an exposure period of 5 days is shown in Figure 16. There was no significant oxidation in milkfat samples exposed to light of wavelengths longer than 443 nm. Exposure of de-pigmented milkfat samples to light of shorter wavelengths, namely, 443 and 410 nm resulted in peroxide value of 1.25 and 1.60 mEq/kg fat respectively compared with 0.36 mEq/kg fat in the control samples. A still greater amount of oxidation was observed in the samples exposed to the near ultraviolet . Similar results were obtained with the de-pigmented milkfat containing added β-carotene and exposed for 6 days (Figure 16). In this case, a greatly increased amount of oxidation was observed in the samples exposed to the

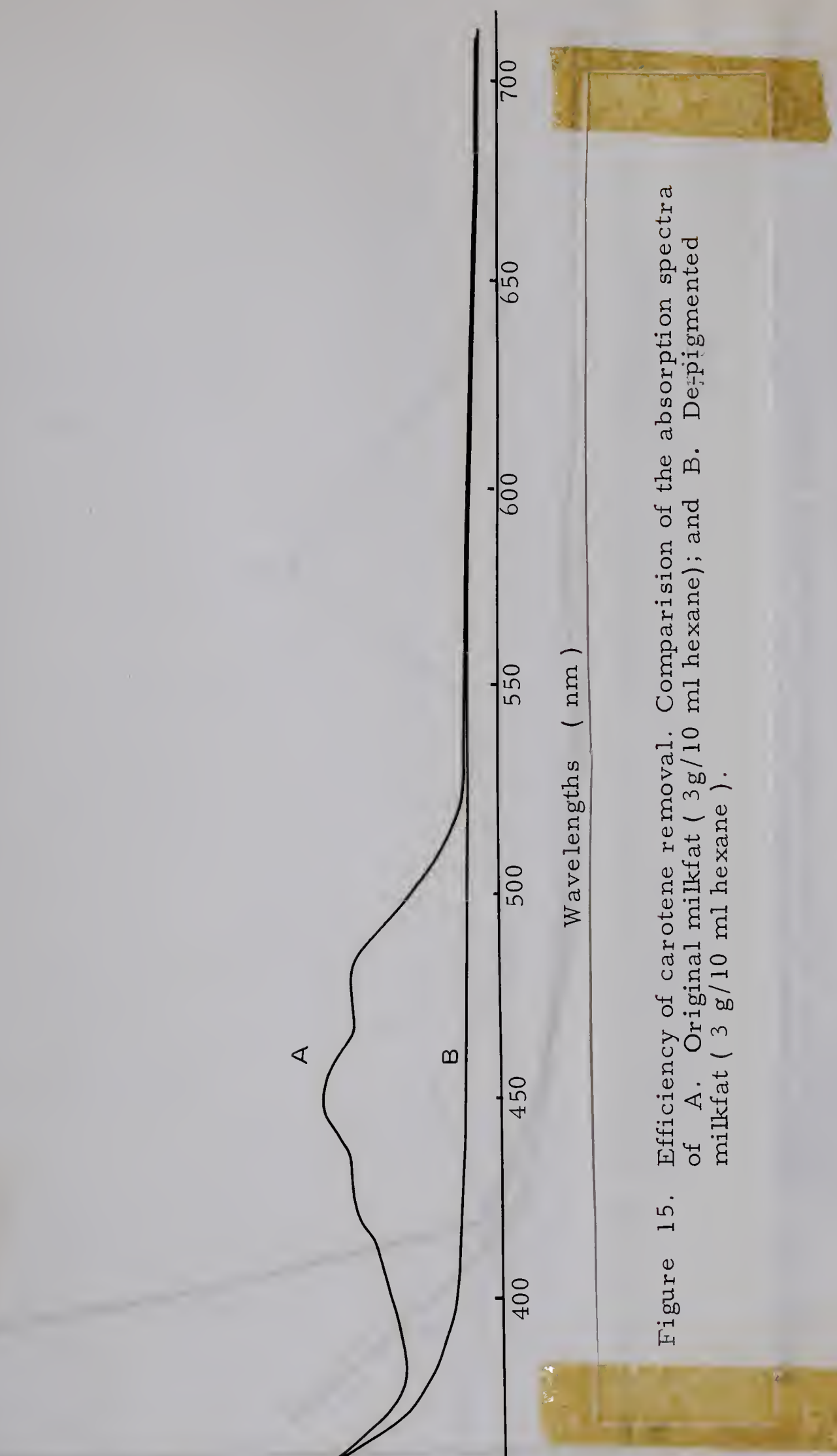

Figure 15. Efficiency of carotene removal. Comparision of the absorption spectra of A. Original milkfat (3g/10 ml hexane); and B. Depigmented milkfat (3 g/10 ml hexane).

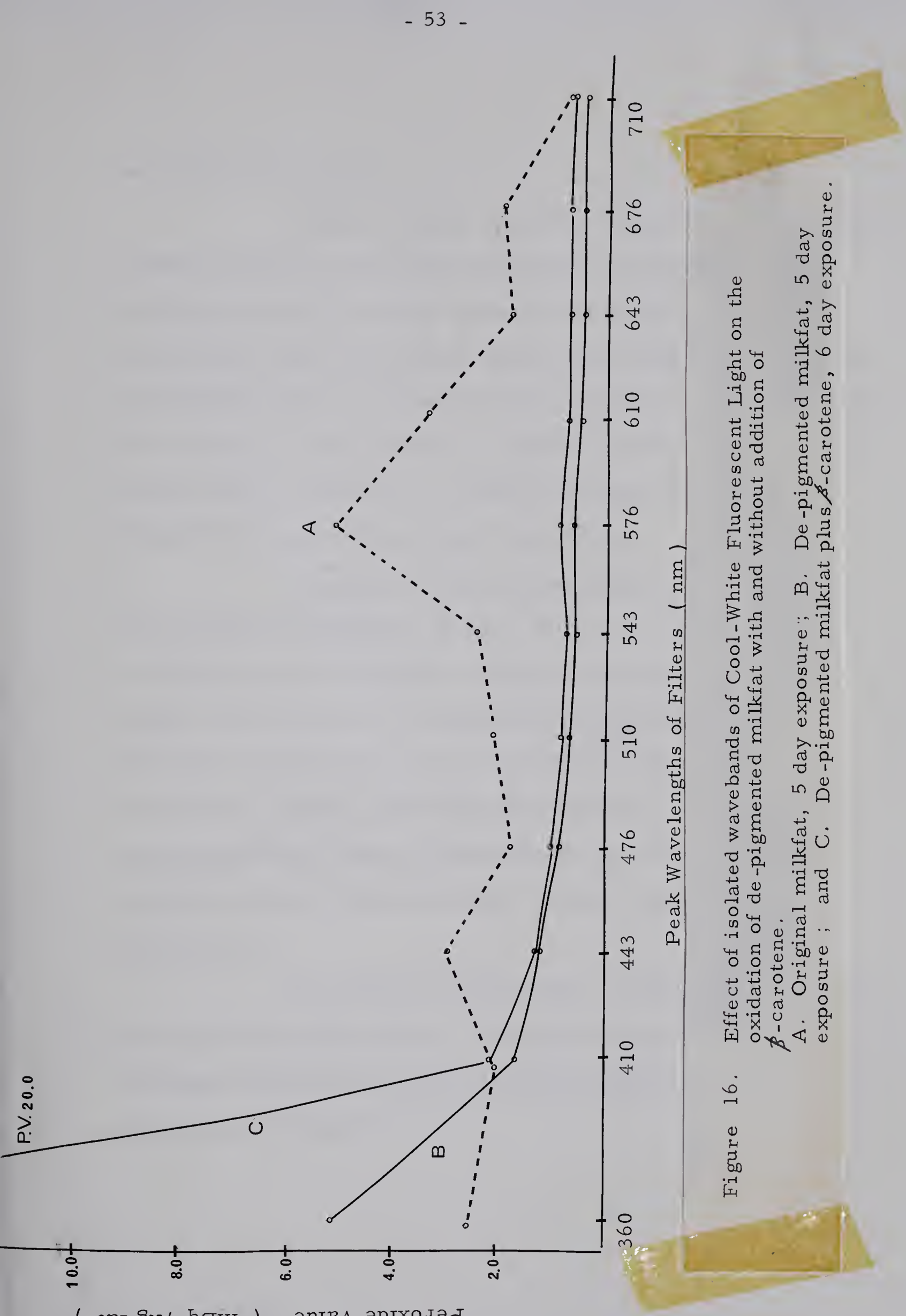

Figure 16. Effect of isolated wavebands of Cool-White Fluorescent Light on the oxidation of de-pigmented milkfat with and without addition of β-carotene.
A. Original milkfat, 5 day exposure; B. De-pigmented milkfat, 5 day exposure; and C. De-pigmented milkfat plus β-carotene, 6 day exposure.

near ultraviolet region.

Figure 17 shows the rate of carotene decomposition compared with the rate of peroxide build up in milkfat samples exposed at a distance of four inches to the whole spectrum of Cool-White Fluorescent Lamp. Under these conditions, increases in peroxide value were observed after only half an hour of exposure. The carotene content, however, remained practically constant for a time while peroxides were rapidly increasing and declined steadily through the later stages of oxidation.

The effect of added phosphatides on the oxidation of de-pigmented milkfat is shown in Figure 18. Appreciable oxidation occured in samples exposed to 410 nm waveband.. Again, as in the case of de-pigmented milkfat (Figure 16), intensive oxidation was observed in samples exposed to near ultraviolet radiation, and among these samples, those containing milk phosphatides showed approximately twice the amount of oxidation that was observed in those containing phosphatidyl ethanolamine.

The degree of unsaturation of depigmented milkfat was not substantially different from that of the original samples, although unsaturation was increased in samples containing added phosphatides (Table 3) .

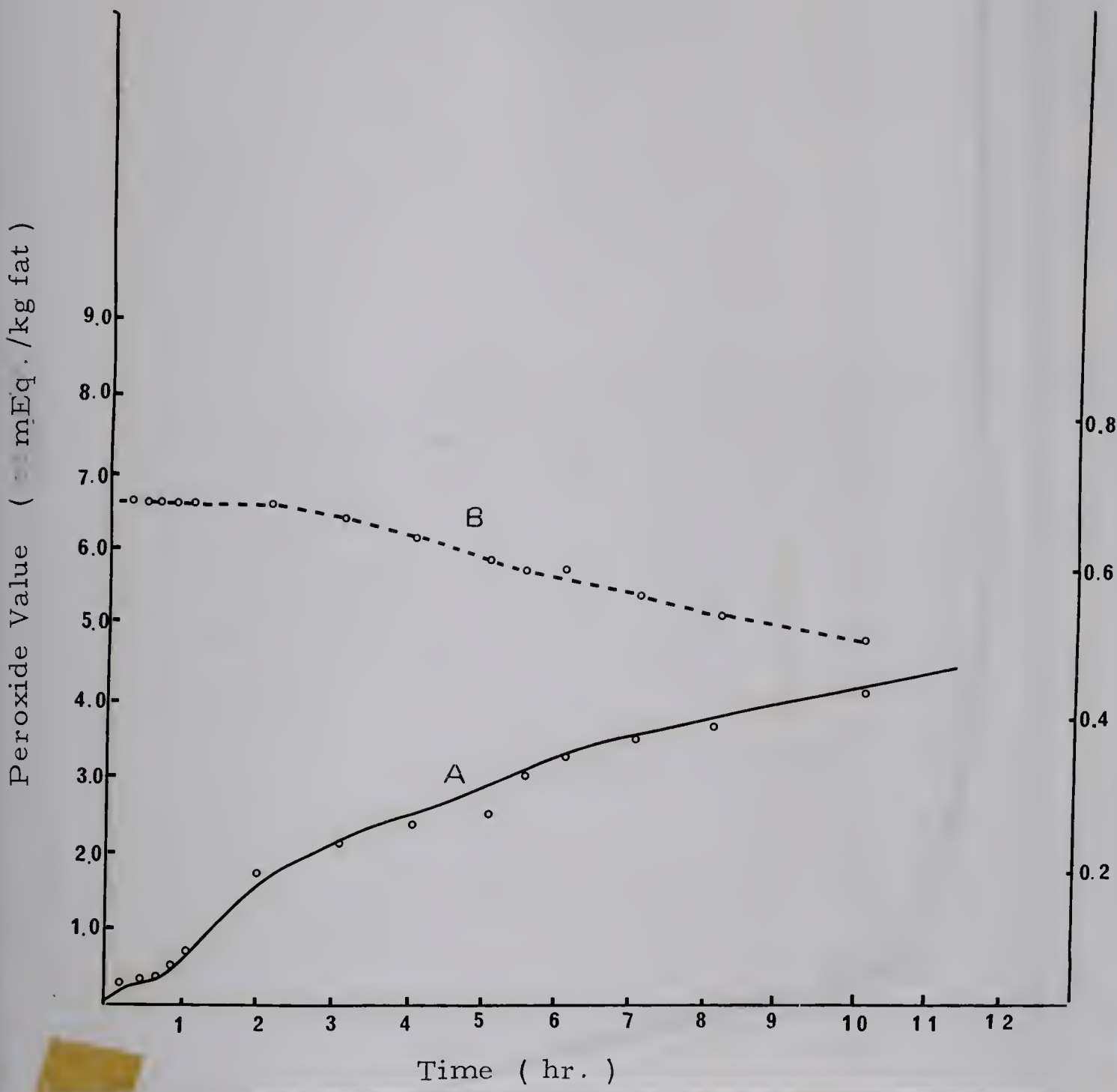

Figure 17. Relationship between peroxide formation (A) and decomposition of carotene (B) under the irradiation of Cool-White Fluorescent Lamp at 4 inch distance.

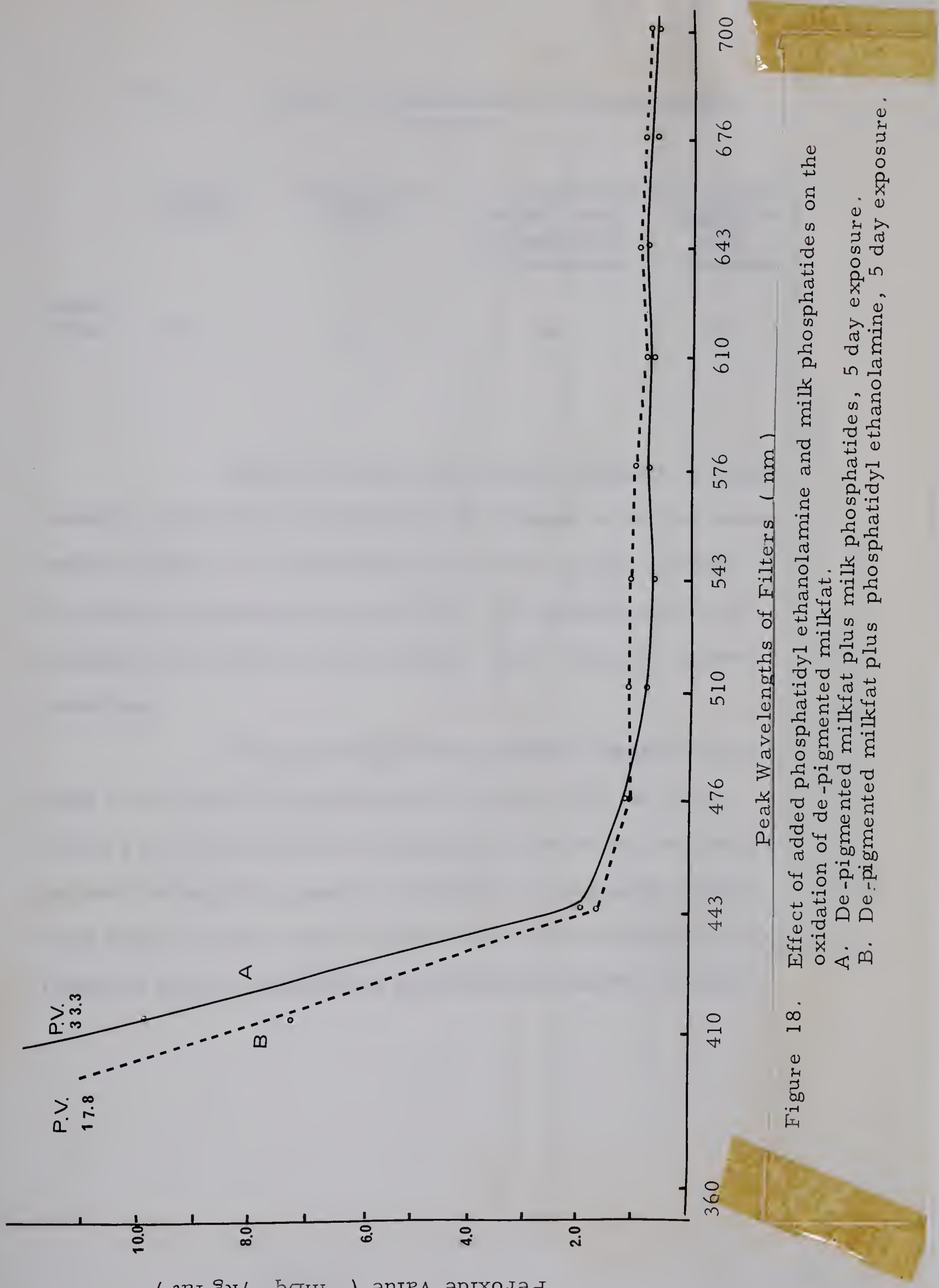

Figure 18. Effect of added phosphatidyl ethanolamine and milk phosphatides on the oxidation of de-pigmented milkfat.
A. De-pigmented milkfat plus milk phosphatides, 5 day exposure.
B. De-pigmented milkfat plus phosphatidyl ethanolamine, 5 day exposure.

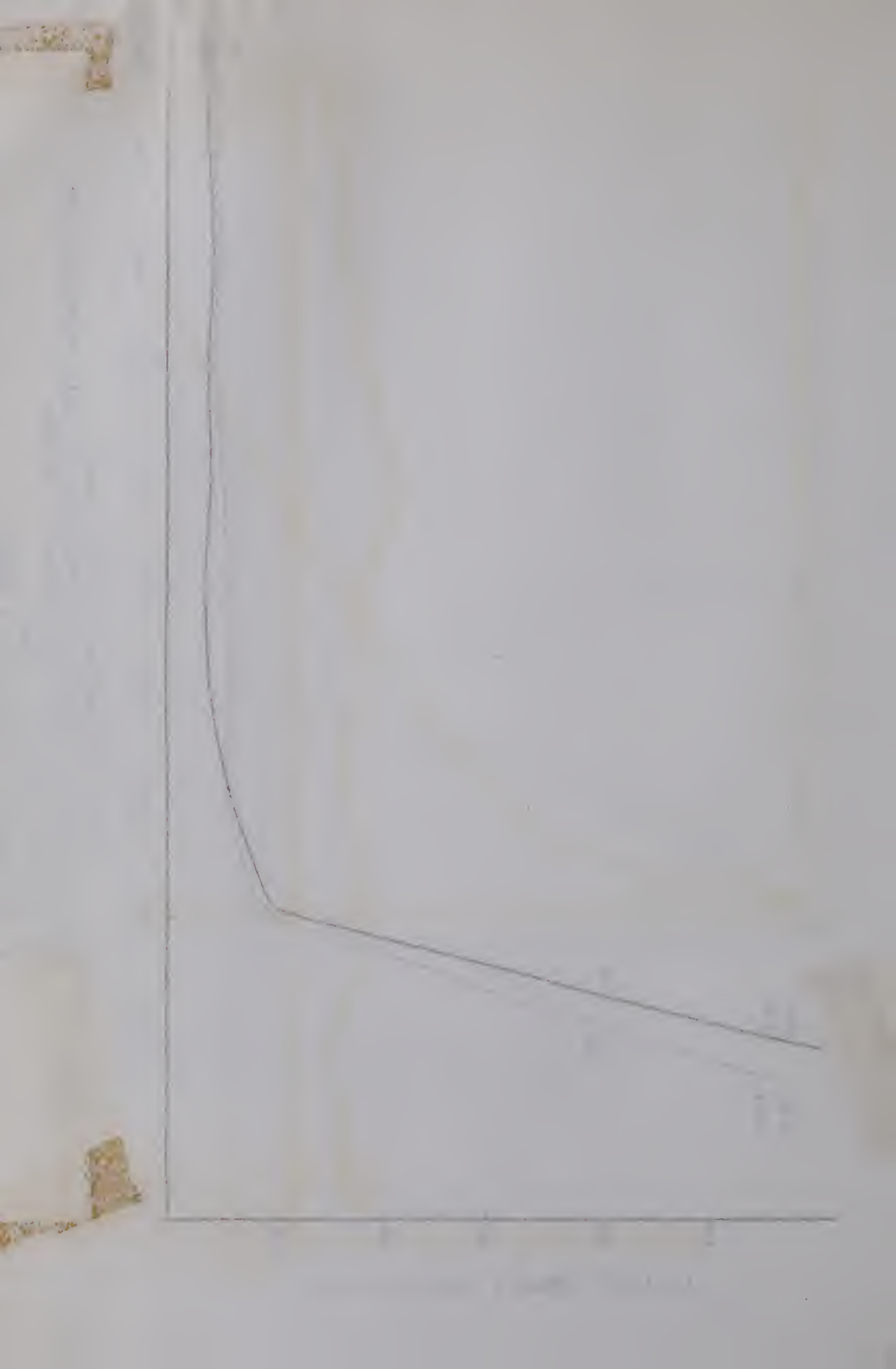

Table 3. Degree of unsaturation of various milkfat samples

	Original milkfat	De-pigmented milkfat	De-pigmented milkfat with phosphatidyl ethanolamine	De-pigmented milkfat with milk phosphatides
Iodine value	25	24	28	31

Figure 19 shows the extent of oxidation in milkfat samples exposed in an atmosphere of nitrogen to narrow wave-bands of light from Cool-White Fluorescent Lamp. Although the extent of oxidation was much less, the general pattern of oxidation was similar to that which is observed under normal conditions.

The rate of oxidation of milkfat exposed only to light of waveband 576 nm is shown in Figure 20. The curve shows a period of slowly increasing peroxide value (an induction period) followed by a period of rapidly increasing peroxides. This shape of curve, typically observed in fat stability studies indicates that the oxidation is probably autocatalytic in nature.

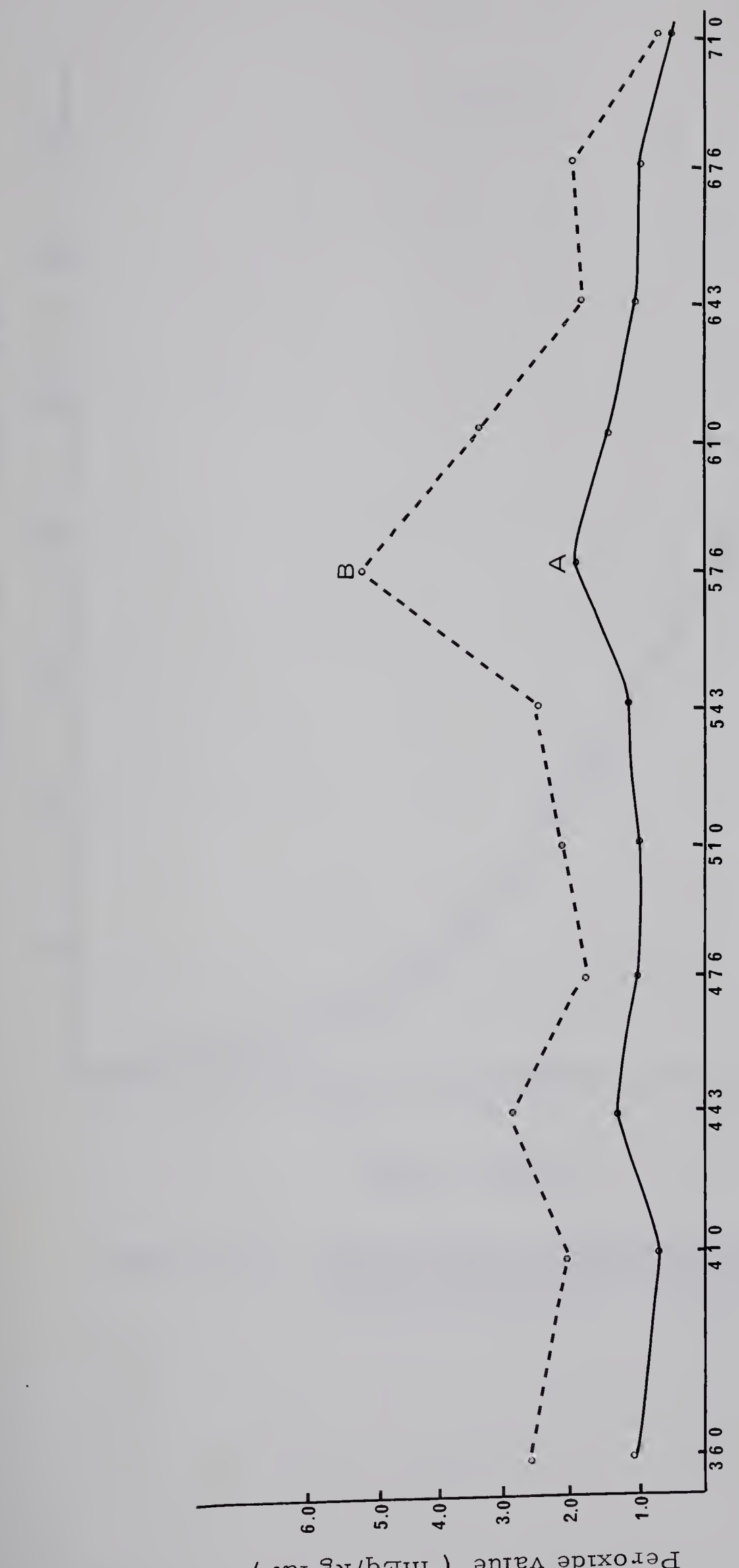

Figure 19. Effect of inert atmosphere on the pattern of oxidation of milkfat exposed to isolated wavebands of Cool-White Fluorescent Lamp.
A. Under nitrogen ;
B. Under normal conditions.

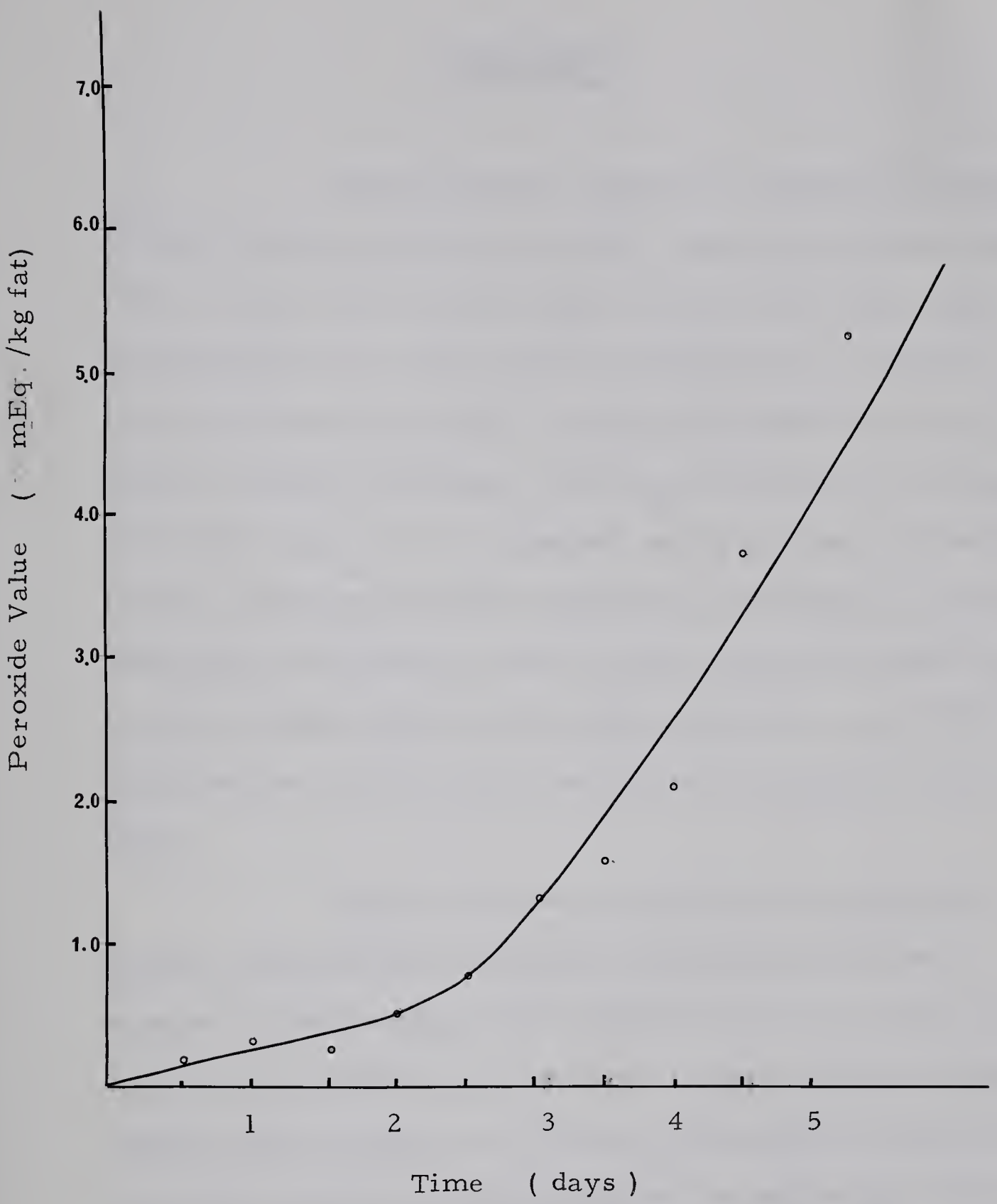

Figure 20. Stability Curve of Milkfat Irradiated with 576 nm Waveband at 7" Distance

DISCUSSION

Benzoyl peroxide appears to inhibit the normal oxidation pattern observed in milkfat exposed to different wave-bands of light from the Cool-White Fluorescent Lamp. It is possible that one or more active components are rendered inactive by benzoyl peroxide, making impossible the normal pattern of milkfat oxidation. The intense oxidation in the near ultraviolet range may be explained, at least in part, by the photolysis of benzoyl peroxide to yield chain initiating free radicals. Photolysis of peroxides is more readily achieved by short wavelengths of visible light and ultraviolet radiation. Lea (1962) explained the proxidant effect of light in fat oxidation on this basis.

Results from the irradiation of de-pigmented samples indicated that all active components present were removed by the treatment with activated charcoal. Failure of the β-carotene addition to restore the normal pattern of oxidation suggests that carotene is not the sole chromophore involved in the normal oxidation of milkfat exposed to visible light. However, carotene is prone to oxidation and its oxidation products might play some role in the dynamic system of fat deterioration. Carotene has been regarded both as a pro-oxidant and an antioxidant. Its role in fat oxidation is much disputed and remains controversal (Emanuel and Yu, 1967). In milkfat

exposed to the full spectrum of Cool-White Fluorescent Lamp, the carotene content showed no significant decrease during the early stages of oxidation. Apparently, peroxides were formed before carotene was significantly bleached or decomposed.

The cause of higher oxidation in the near ultraviolet range after the addition of -carotene might be due to the photolysis of both carotene oxidation products, and the small amounts of peroxide formed during sample treatment. Autoxidation of polyunsaturated fatty acids leads to the production of conjugated unsaturated system, which absorb ultraviolet light at certain wavelengths (Lundberg, 1962). In such systems, ultraviolet light markedly accelerates the decomposition of peroxides, and may also influence the course of normal autoxidation reactions by other mechanisms.

Addition of pure phosphatidyl ethanolamine and milk phospholipids to the de-pigmented milkfat gave rise to oxidation patterns similar to that observed in de-pigmented milkfat with added carotene , except that the samples exposed to ultraviolet light were oxidized more extensively. Again the almost complete absence of oxidation in samples exposed to wavebands longer than 443 nm indicated that all active chromophores or constituents had been removed from the system.

The autocatalytic nature of milkfat oxidation was

indicated by the oxidation of milkfat samples in nitrogen atmosphere and the induction type of curve obtained in the oxidation of milkfat exposed to the 576 nm waveband. Autoxidation of fat is generally characterized by a typical curve, the slope of which, increases as the oxidation progresses. The rate of oxidation is also depended upon oxygen partial pressure. The slight oxidation observed in inert atmosphere might be attributable to residual oxygen left either in the sample or in the glass chamber used.

The normal pattern of oxidation in milkfat exposed to light of different wavebands indicates the probable existence of one or more components present in small amounts in milkfat, difficult to detect spectrophotometrically, and removable by treatment with activated charcoal. The presence of these trace components characteristically influences the early stages of the oxidation of milkfat exposed to isolated wavebands of fluorescent light. In the absence of these substances, oxidation is promoted by exposure to light of the ultraviolet range but unaffected by exposure of the visible region.

If further investigation is to be made, it would be of interest to study the absorption characteristics of milkfat by reflectance measurements of the fat surface in the course of oxidation. It should be emphasized that the area of greatest change is the exposed surface. Reflectance spectra obtained

from samples exposed to various wavebands or for various length of time might not only provide information on the change of absorption pattern on the substrate surface, but also indicated to what extent various wavelengths of light are absorbed by milk-fat.

Measurement of thermal effect on solid fat surfaces in the course of exposure might present some experimental difficulties. However, this is also one possible avenue in proving whether a thermal effect plays a role in the photochemical oxidation of solid fat.

The nature of the unknown constituent or constituents through which the visible light is able to exert effects to promote oxidation especially deserves further study. Of particular difficulty is the isolation in pure form of the active constituent or constituents, since their chemical nature is virtually unknown. Extraction of the active constituents from the charcoal used to remove carotene furnishes a possible way to study the unknown components.

Much remains to be learned regarding the effect of visible light on the oxidation of milkfat and many experiments will be required to permit a description of the reactions involved.

SUMMARY

The addition of benzoyl peroxide to milkfat greatly increased the extent of oxidation observed in samples exposed to the near ultraviolet portion of the fluorescent light spectrum, whereas oxidation in samples exposed to the visible region was reduced. It is suggested that the increased oxidation could be attributed to the photolysis of peroxides by shorter wavelengths of light.

Treatment of milkfat with activated charcoal greatly changed the pattern of oxidation observed when milkfat was exposed to different wavebands of light from Cool-White Fluorescent Lamp. The oxidation maximum shifted from the usual 576 nm waveband to the near ultraviolet. The addition of β-carotene failed to restore the original oxidation pattern, suggesting that carotene was not the only component involved in the photochemical changes that normally occured.

Additions of pure phosphatidyl ethanolamine and milk phosphatides to the charcoal treated milkfat also did not restore the normal pattern of oxidation in milkfat exposed to visible light but again caused a marked increase in the extent of oxidation observed in samples exposed to the violet and near ultraviolet part of the light spectrum. The degree of unsaturation appeared not to affect the extent of oxidation in de-pigmented

milkfat samples exposed to visible light.

Evidence obtained from the oxidation of milkfat samples exposed to light in inert atmosphere and the induction type of curve observed in the oxidation of milkfat exposed to a single waveband suggest that the photochemical reactions might be autocatalytic in nature.

APPENDIX

STUDIES ON THE THIOBARBITURIC ACID TEST FOR USE IN MEASURING MILKFAT OXIDATION

General Introduction

The thiobarbituric acid (TBA) test is widely used for measuring oxidative changes in foods containing unsaturated fatty acids. There are various modifications and procedures of the test, most of them being concerned with the adjustment to the particular fats or lipids under investigation. The various modifications proposed for use with different fats and different food products may be grouped under two headings : refluxing methods and distillation methods.

Refluxing methods: In these methods, an acid solution of 2-thiobarbituric acid is added to the fat or fat-containing foods and the mixture is refluxed in a waterbath for periods ranging from 10-50 minutes. The pink pigment formed in the reaction is extracted with a suitable solvent or solvent systems and measured in a spectrometer (Briggs and Bryant, 1953 ; Turner et al. 1954 ; Sinnhuber and Yu, 1958.).

Distillation methods: The fat is first steam-distilled with acid, and the TBA-acid solution is added to a portion of the distillate, which is then heated in a waterbath for maximum color development. The pigment is measured

directly in a spectrometer without the use of extraction solvents (Sidwell, Salwin and Mitchell, 1966 ; Tarladgis , Watts and Younathan, 1960.).

Published TBA test for milkfat employed the refluxing procedure (Patton and Kurtz, 1951 ; Briggs and Bryant, 1953.) and that of Briggs and Bryant is most commonly used.

In the work reported here, various modifications of the foregoing methods have been investigated in order to devise a procedure suitable for routine tests on milkfat. The procedure proposed is essentially that of Briggs and Bryant with modifications that have been introduced for use with other fats (Sinnhuber and Yu, 1958 ; Yu and Sinnhuber, 1967.) :

1. Reduction of reagent volume to bring the resulting pigment absorbance into the photometrically accurate range of spectrometers.
2. Reduction of the effect of air oxidation during the test by adding an antioxidant mixture to the reaction medium and coating glassware with a water-soluble silicone oil.
3. Expression of experimental results in terms of TBA number (mg malonaldehyde/kg fat).

TBA Procedure

Reagents

TBA reagent : 0.025 molar 2-thiobarbituric acid in molar phosphoric acid.

Isoamyl alcohol.

Pyridine

Extraction mixture : 2 : 1 mixture of isoamyl alcohol and pyridine.

Emulsifying reagent : Myvatex food emulsifier, type 8-20 (Distillation Products Industries, Rochester, N.Y.)

Antioxidant mixture : 100 mg of trihydroxybutyrophenone (K & K Laboratories Inc., Plainview, N.Y.) dissolved in 1.8 g of propylene glycol. 100 mg of Tenox II (Tennessee Eastman, Kingston, Tennessee) added.

Siliclad : A water-soluble silicone concentrate (Clay-Adams, Inc., N.Y.)

Apparatus :

Spectronic 20 : A spectronic 20 spectrometer is used for all absorbance determinations.

Waterbath : The TBA reaction is carried out in a constant temperature waterbath at 98 ^{o}C (boiling water temperature).

Rack : A rack is required to hold a series of suitable reaction vessels.

Reaction vessels : The reaction vessels may be either 15mm O.D.x 150 mm screw-cap test tubes, or 125 ml ground glass-joint Erlemeyer flasks fitted with a standard West type condensers.

Clean test tubes are filled with 1% (v/v) diluted Siliclad solution, allowed to stand for ten seconds, emptied, rinsed immediately with tap water, and then with distilled water. The tubes are dried at 100°C for 2 hours.

After the TBA test, the tube or flask is rinsed with acetone and water. The tube is then placed in a boiling detergent bath containing 100 g of detergent and 20 g of NaOH dissolved in 2 liters of water for 20 minutes. The tube is taken out and soaked in dilute HCl solution (0.36 %) for 10 min. After thorough rinsing with water, the test tube is ready to be recoated.

Procedure

One to three grams of milkfat is placed in the bottom of a screw-capped test tube by means of a medicine dropper and weighed. Five drops of antioxidant mixture is then added followed by approximately 0.2 g of emulsifier. Twenty milliliter of acid TBA reagent is transferred by pipet to the test tube, which is then placed in a rack and heated in a boiling waterbath for 5 minutes (with the plastic cap screwed on). The tube is removed from the bath and the contents are mixed on a Vortex mixer and placed back in the boiling waterbath. After heating for another 30 min ,

the tube is cooled to room temperature in a cold waterbath. A glass rod is used to either remove or to make a hole in the floating solidified fat layer to permit the insertion of a 10 ml pipet. A 10 ml aliquot of the reaction mixture is then transferred to a 40 ml centrifuge tube which contains 10 ml of extraction solvents. The contents are mixed and centrifuged at 2,500 rpm for 5 minutes. An aliquot of the upper layer solvents is transferred to a cuvette for measurement of absorbance at 535 nm.

The result is expressed in terms of the TBA number which is calculated as follows :

$$\frac{\text{Net absorbance at 535 nm}}{\text{Sample weight (g)}} \times 12$$

Reagent volume and sample size . Of the various reagent volumes tried, 20 ml appeared to give the best results. The amount of TBA reagent contained in 20 ml is sufficient to react with the amount of malonaldehyde normally present. Similar results were obtained when larger reagent volumes were used and theoretical calculations indicated that the amount was more than sufficient for the reaction.

Using 20 ml of the reagent mixture, the relationship between sample size and absorbance was linear for samples of one to three grams of milkfat. With intensely oxidized samples, use of a small sample size is recommended because with this solvent system, extraction of the chromophore is incomplete.

Air oxidation during the test. Briggs and Bryant (1953) indicated that when the TBA reaction was carried out in inert atmosphere, it gave results that were lower than those obtained under ordinary conditions because of the oxidation of the substrate occured during the test. However, carrying out the reaction in an inert atmosphere is cumbersome for routine use and the TBA test is usually performed under atmospheric conditions. When the precautions (coating glassware with a water-soluble silicone oil and adding a mixture of antioxidants to the reaction medium) proposed by Yu and Sinnhuber (1967) were applied, oxidation of the substrate was retarded. Figure 21 shows a comparision of the TBA reaction conducted under ordinary conditions (A) and glassware coated, antioxidants added mixture (B). High values were observed in all cases for the samples analyzed under ordinary conditions and the differences were more pronounced with more extensively oxidized samples. Moreover, in determinations on samples treated under ordinary conditions the absorbance did not level off at the end of the heating period but continue to increase whereas, when samples were heated under the modified conditions, the absorbance remained steady at the end of the heating period.

Derivation of the TBA constant. The TBA constant is derived from a standard curve (Figure 22) prepared by using TEP (1,1,3,3-tetraethoxypropane, Eastman Organic Chemicals, Rochester, N.Y.).

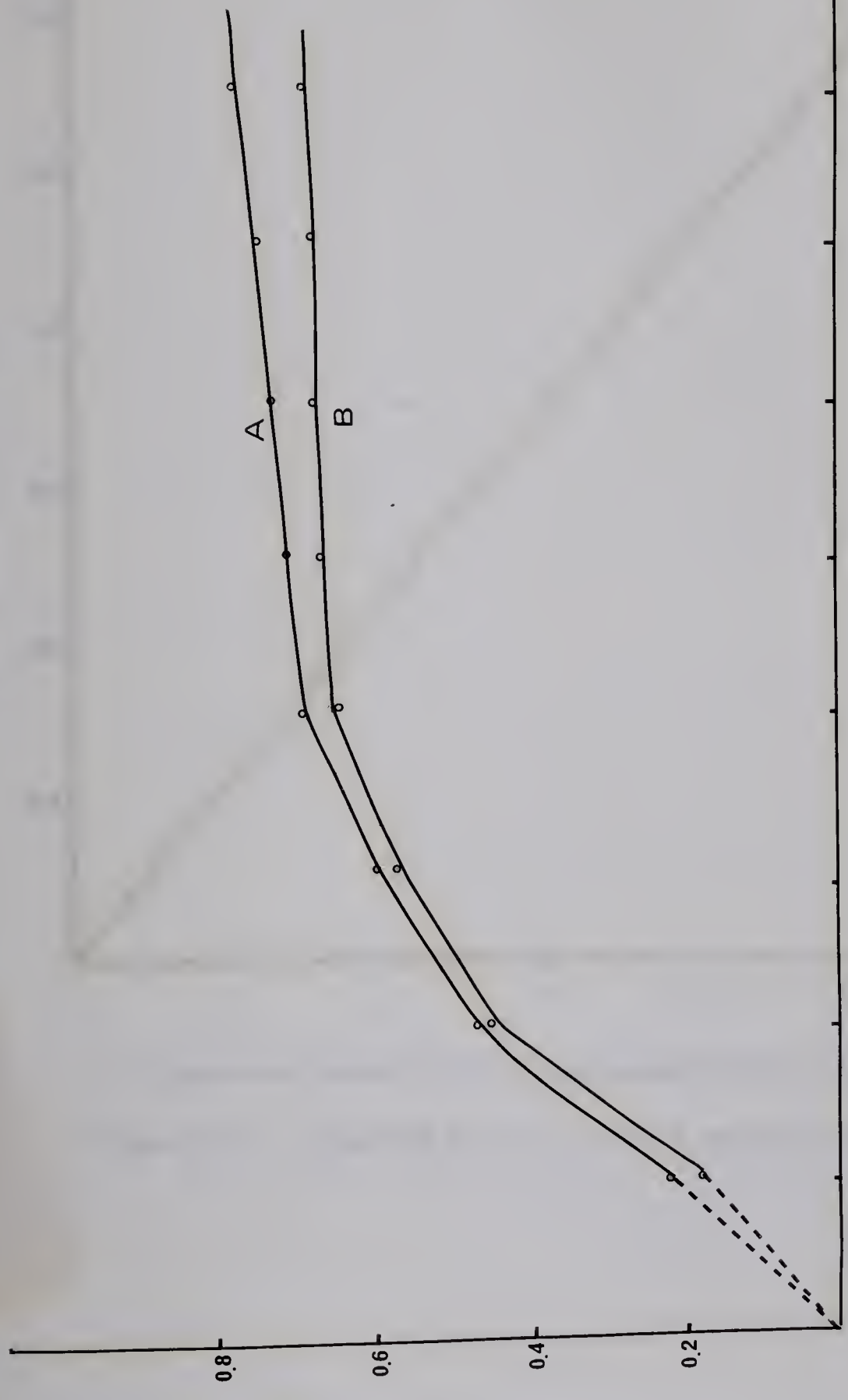

Figure 21. Comparision of TBA Reactions with Oxidized Linoleic Acid Carried Out at Two Different Conditions. A. Ordinary conditions. B. Silicone-coated glassware plus antioxidant mixture.

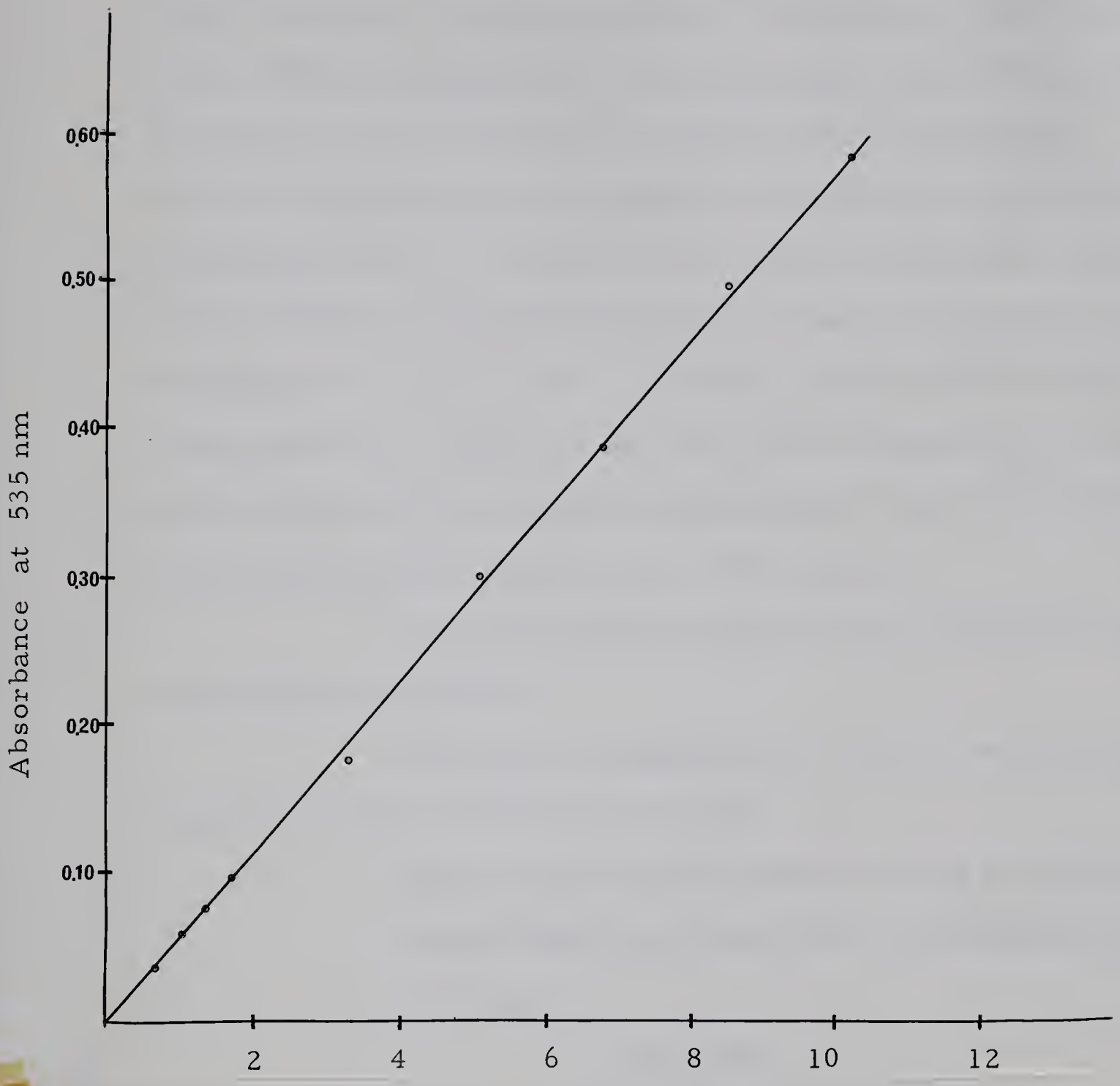

Figure 22. Standard curve of TEP (malonaldehyde)

The standard curve was obtained by performing the TBA reaction with various amounts of TEP which, on acid hydrolysis, yields malonaldehyde on an equal molar basis. The procedure was altered only by reducing the heating time to 20 minutes because maximum color development occured more rapidly in this monophase system. It should be noted that the intensity of absorbance of TBA-malonaldehyde complex is lower in a pyridine solution than it is in an acid one. The TBA constant derived from other procedure (Sinnhuber and Yu, 1968; Tarladgis et al. 1960.) involved the measurement of the chromophore in an acid medium and therefore gave rise to smaller TBA constant.

From the obtained standard curve, the TBA constant was calculated as follows :

Absorbance resulting from 1 mole of malonaldehyde in 20 ml reaction medium is 5,910,000.

Since 1 mole of malonaldehyde = 72 g or 72,000 mg, the absorbance resulting from 1 mg malonaldehyde in 20 ml is

$$\frac{5,910,000}{72,000} = 82.1$$

and since,

$$\frac{\text{absorbance of 20 ml reaction mixture}}{\text{sample weight (g)}} \times 1000$$

is the absorbance at 535 nm from 1000 g sample,

$$\frac{\text{absorbance of 20 ml reaction mixture}}{\text{sample weight (g)}} \times \frac{1000}{82.1}$$

= mg malonaldehyde/kg fat

The term $\frac{1000}{82.1} = 12.1$ or approximately 12

Thus 12 is referred as the TBA constant for a 20 ml reaction mixture.

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